TRIBUTE TO DR. H. GIDEON WELLS,
INVESTIGATOR, SCHOLAR, TEACHER (MAGISTER)

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When it was suggested that I write a few words about Dr. Wells, my teacher and colleague, diffidence nearly deterred me from accepting this rare privilege for even under the most favorable conditions I would feel unequal to this otherwise happy assignment. On the other hand, honorable recognition of distinguished *living* contributors to human welfare is, on the whole, unfortunately so rare that any mite must be offered which will foster a custom which should be observed more commonly. In attempting this, there looms up one obvious difficulty in that the resulting effort may appear too much like an obituary notice or an "In Memoriam." No such difficulty can arise in the present instance, however, for Dr. Wells is still much alive and active, and though retired from active duty at the University, still evinces that exuberance of spirit and that sense of humor which it was my good fortune to discover, appreciate and profit by when I first met him 36 years ago.

The detailed academic "Vita" of Dr. Wells can be found by the interested reader in any copy of "American Men of Science." More to the point is the printed bibliography of his published writings which lies before me, comprising twelve typewritten pages of titles of articles of diverse nature and published in a variety of journals. These began in 1897 and have continued down to 1940; and they range from the expected Virchowian pathology to the biochemical aspects of physiologic and pathologic processes and "Education in Hospital and Laboratory." As time went on, the emphasis was more and more on the biochemistry of disease and immune reactions, particularly as occurring in calcification and tuberculosis. Malignant neoplasms had always attracted the attention of Dr. Wells, and since Maud Slye became associated with him, his interest has centered in that direction for more than 2 decades. As director of this work from the beginning and as able counselor to Miss Slye and Miss Holmes, Dr. Wells with great

magnanimity, as Chief of the Laboratory, never took advantage of their arduous and brilliant work.

Of the three books written by Dr. Wells—one of which was translated into German and French—the most important, which passed through five editions (the last in 1925), is his monumental volume of 790 pages on "Chemical Pathology." A pioneer in its field, this comprehensive book was as original and as important as was Claude Bernard's "Physiologie Générale." The other two volumes are special extensions of chemical pathology as it pertains to tuberculosis and immunity. In this field Dr. Wells was "bahnbrechend," not only for his encyclopedic collection of available data but for his own biochemical contributions. This work constitutes more than any other his monument to lasting fame.

In his modest way, Sir Charles Bell boasted that in his lifetime he had, as teacher of anatomy, neurology and surgery, taught some 700 to 800 medical students. Considering the times this certainly constituted a huge number since pupils then chose their instructor rather than being assigned to a teacher by circumstance. Even so, I know of no distinguished pupils of Charles Bell, such as Wells's Harry Corper, Maud Slye, Esmond Long and Paul R. Cannon. All are productive scholars and teachers. I have no doubt that had Dr. Wells taught in the times of Bell, his students would have equalled, if not exceeded, in number those of his predecessor of a century before; for of all the teachers I have had Dr. Wells was the best, with the late Dr. Julius Stieglitz a close second. Perhaps I can sum up the matter best by altering an old Latin adage: "Magister seu poeta nascitur; orator fit."

Wells, a born teacher, was never an orator but his good humor in the classroom and his "wise-cracks" or "gags" helped much to keep the classes on the alert. I happened to be in his first autopsy course together with Harry Corper, Robert L. Benson, Clyde Brooks and Esmond Long. Well's unique manner of quizzing instead of lecturing was most effective in inculcating into us and into thousands of other students the rudiments of pathology. It took a master in pedagogy to use this method effectively. Others have tried it but few have achieved more than mediocre success.

At this happy moment when his colleagues in his own field welcome this special number of the *American Journal of Pathology* in honor of Dr. H. Gideon Wells, his more intimate associates rejoice in having known personally this fine investigator, superb teacher, generous executive and appreciative friend.

EPITHELIAL METAPLASIA IN CONGENITAL CYSTIC DISEASE OF THE LUNG *

Its Possible Relation to Carcinoma of the Bronchus

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As primary cancer of the lung is becoming more and more frequently an operable lesion, opportunities to observe earlier growths are likewise becoming more frequent. This is of greatest importance for it affords a chance to correlate the microscopic picture, as determined from bronchoscopic biopsy, with the gross pathological findings in the early stages of the disease. In such a way a confident understanding of the clinical picture may be reached. Before this understanding can be brought about, however, such a correlation between the microscopic and clinical picture must be established.

As was suggested several years ago,[1] forms of classification based on the microscopic picture as seen in far advanced lesions have but little to offer the clinician. Many of the tumors, by the time they have become widespread, exhibit a marked degree of pleomorphism producing different phases of cellular differentiation depending upon the site from which the section was taken. However, this does not always occur, for at times widespread tumor growths will be seen in which sections taken from many areas will show the same phase of cellular configuration.

There seems to be but little correlation between the amount of cellular differentiation and the extent of the growth. Squamous cell cancer of the bronchus offers a good example of this. It is not unusual to find a very well differentiated squamous type of tissue in an early lesion; nor is it unusual to find a similarly well differentiated type of squamous epithelium in a far advanced lesion. The same lack of correlation between the amount of cellular differentiation and extent of growth holds true in squamous cell cancer that shows even poor differentiation. We have on numerous occasions seen relatively poorly differentiated squamous epithelium in lesions definitely operable. In view of the fact that normal bronchial epithelium under certain conditions possesses the ability to form keratin, it is difficult to interpret

645

the meaning of keratinization in carcinoma of the bronchus and the relationship to its ability to spread. Until such an interpretation is forthcoming its clinical significance must remain vague. It seemed to us, therefore, that any correlation between a particular microscopic picture and the extent, and therefore the operability, of the lesion is so often hazardous that various morphological classifications based on the microscopic picture of the lesion are all of but little clinical value.

Recently we had reason to modify the foregoing conceptions somewhat.[2] As our experience with early lesions increased we began to encounter a type of tumor that could be set apart from the more classical type of bronchiogenic carcinoma. It was associated with a very definite clinical picture. It was seen in females just as often, if not oftener, than in males. It usually occurred in younger people and often was characterized by rather long-standing clinical features, many of the patients giving symptoms dating back a number of years before more active growth had occurred. The pathologic picture was different from the usual type of carcinoma in the sense that the lesion protruded into the bronchus as a nodule covered with epithelium. This epithelium was generally squamous and overlay an area of fibrous tissue distal to which tumor cells were seen. These tumor cells were often arranged in cords and alveoli resembling unaërated fetal lung tissue. Not infrequently there also was found evidence of mesodermal tissue such as cartilage, bone, muscle and often an extreme vascularity. When the specimen along with the entire lung or lobe was removed surgically it was noted that the tumor was often collar-button-shaped. The larger portion of it was frequently outside the lumen of the bronchus extending into the lung parenchyma. The fact that only a minor portion was evident and the major portion invisible suggested the analogy to an iceberg. It may or may not have metastasized. Almost universally there was some type of congenital abnormality of the lung tissue. This was present most frequently as an abnormal number of lobes. Other pulmonary malformations were observed. Because of the resemblance to fetal lung tissue, its association with congenital abnormalities of the lung and the mixed tissue elements present, it occurred to us that this lesion was probably one intimately connected with congenital malformation of the lung.

This conception then would recognize two main types of bronchial tumors. One of these arises in apparently adult epithelium, is much more frequent in the male after middle age, usually possesses some tendency on the part of the epithelium to form keratin and generally spreads fairly rapidly. The other type probably arises in tissue of embryonic type, is generally associated with congenital pulmonary disorganization, does not favor either sex and may remain in the same site for many years before invading other tissue. Each of these types, therefore, presents features both morphologic and clinical that make it distinguishable in most instances. Such a classification has been of the greatest value to us from the standpoint of both therapy and prognosis. The latter type includes the tumor which in the literature is commonly designated as bronchial adenoma. In our opinion this tumor should be regarded as potentially malignant, although in many instances it may show no tendency to invade other tissues for many years and doubtless occasionally has been successfully removed through the bronchoscope.

We have been particularly interested in those tumors often associated with developmental defects in the lung.

If such a conception is true, in all probability one should occasionally be able to find evidence of epithelial overgrowth in pulmonary tissues which are the site of congenital malformation. The following report is concerned with efforts to identify such evidence of overgrowth in the epithelial elements of disorganized pulmonary tissue before such overgrowth was evident clinically.

OBSERVATIONS

Because of the availability of surgical material, congenital cystic disease of the lung was taken as a lesion evidencing abnormal pulmonary formation. All of this material presented severe secondary infection with concomitant distortion of the anatomical picture. Accordingly, only those cases were used in which adequate, careful studies could be made. There were 9 such patients, 6 of whom were treated by total pneumonectomy and 3 by lobectomy.

Immediately after the surgical removal of the lung or lobe the specimen was distended by the injection of Kaiserling's solution into the patent bronchi, care being used not to produce over-

distention and distortion. After fixation for 24 hours the specimen was sectioned along the axis of the major bronchi. A longitudinal slab of the entire lung or lobe, as the case might be, approximately 1 cm. thick, was then made and this was cut into smaller blocks which were then fixed in formaldehyde for 24 hours and sectioned after paraffin embedding. The sections were stained with hematoxylin and eosin, phosphotungstic acid and hematoxylin, and, in instances where indicated, with Mallory's aniline blue and orange G stain. The sections were studied particularly in relation to the normal architecture of the bronchi and bronchioles with attention to the relative amount and type of tissue going into the formation of these structures.

Of the 9 specimens submitted to such study, in 3 there was evidence of epithelial overgrowth found microscopically which upon subsequent examination of the gross specimen was not apparent with the naked eye. In none of the specimens was any gross evidence of tumor formation seen. It must be assumed, therefore, that the structures to be described are entirely local in their growth.

REPORTS OF CASES

Case 1

A. V., male, age 34. Five years before admission to the hospital the patient had suffered an attack of bilateral bronchopneumonia. He was in bed 1½ months and spent an additional 2 months in convalescence. Two years before admission to the hospital he developed a cough which became progressively worse and was productive of white, foul sputum, particularly in the morning. There was a gradual development of dyspnea. Occasionally the sputum was blood-tinged and occasionally there was slight pain in the left chest. Upon admission to the hospital he was found to have numerous cavities throughout the entire left lung which upon bronchoscopic examination were found to be filled with pus. There was no evidence of bronchial obstruction nor of tumor formation. The right lung was normal. Accordingly, on April 3, 1939, the entire left lung was removed by Dr. Brian Blades.

Gross Pathology. Figure 1 shows the gross appearance of the lung. The visceral pleura was covered by firm, fibrous adhesions. Palpation of the lung revealed atelectasis in the lower lobe with many spotty, cystic areas more noticeable in the upper lobe. On sectioning the lung, the pleura was found to be thickened to the extent of about 4 mm. The bronchi and bronchioles, particularly of the upper lobe, extended to large cavities lined by

walls of varying thickness depending upon the amount of inflammation present. Within these cysts there was found a great deal of pus. The lower lobe for the most part was atelectatic although here and there one could see fairly large cysts.

Microscopic Pathology. Very little lung parenchyma was encountered. The interalveolar septa were markedly thickened, presumably as the result of chronic inflammatory change. The alveoli all seemed to be considerably dilated. The cystic cavities were lined by bronchial epithelium, some of which was ciliated and beneath which one found a chronic inflammatory reaction, nonspecific in type, with complete absence of many other bronchial structures. Very seldom was cartilage found. Smooth muscle for the most part was absent. There were no mucous glands. Whether these structures were never formed or whether they had been destroyed by inflammation cannot be determined; the latter would be most unusual. In one area there were noted masses of epithelial cells tending to be squamous in type which had delimited external borders and which were normally differentiated and showed no evidence of mitotic figures. Figure 2 is taken through this area. These cells had their origin well away from bronchial tissue. Although the cell masses were not encapsulated, they gave the appearance of very slow growth.

In the larger bronchi the structures were for the most part normal. Sections through the lymph nodes showed only chronic inflammatory change. The picture throughout all of these sections was that of a congenital lesion of the lung in which the bronchi and bronchioles were enormously dilated into cyst formation. Because of the size of the cysts and because they were lined by perfectly normal bronchial epithelium and showed rather minimal inflammatory change as compared to an unquestioned case of bronchiectasis, one is justified in calling this congenital cystic disease of the lung rather than a marked bronchiectatic change.

Case 2

L. F., male, age 48. Fifteen years before admission to the hospital the patient developed a productive cough which persisted for 2 years and which came on spontaneously. There was gradual improvement. About 2 years before admission to the hospital the patient developed influenza and following his recovery there was a return of the cough, which has remained constant and productive. The sputum was purulent and foul and was much

more profuse in the morning. During these 2 years he had lost approximately 15 lbs. Upon admission to the hospital he was found to have numerous saccular dilatations involving the entire left lung, which upon bronchoscopic examination were found for the most part to be filled with pus. The right lung was relatively normal. There was no evidence of bronchial obstruction of any sort. On April 27, 1939, the entire left lung was removed (E. A. G.).

Gross Pathology. The material examined consisted of the entire left lung. There were many adhesions involving the pleura which were unusually firm. On cut section the bronchi were seen to be tremendously dilated and extended into large cystic cavities which were filled with pus. The lingula was the only portion of the upper lobe involved in the cystic process. The lower lobe was markedly involved and in many areas presented atelectasis.

Microscopic Pathology. Microscopic study showed the bronchi to be markedly dilated. The lining epithelium was everywhere intact and in some places changed to a squamous type. The bronchi in many places were dilated and in most areas presented a notable absence of some of the normal mesodermal elements of the bronchial wall, notably cartilage and smooth muscle. In other areas, however, there was an overabundance of smooth muscle. There was very little scarring or fibroplasia of the bronchial wall and a relatively small amount of infiltration by inflammatory cells. These for the most part were lymphocytes and plasma cells. In the lumen of the bronchi, however, there was considerable pus. Upon studying the extension of the bronchi into the periphery of the lung, cystlike structures lined by bronchial epithelium were encountered. The air sacs were often markedly dilated while in other areas considerable atelectasis was present. An interesting feature around some of the bronchial walls was an ingrowth of the epithelium of the air sacs into the wall itself forming small cystlike cavities. In several areas nests of epithelial cells were observed in the bronchial wall just beneath the epithelium. For the most part these cells were cuboidal, although here and there they showed a tendency to become spindle-shaped, apparently growing at random (Figs. 3 and 4). There was no encapsulation. The nuclei for the most part were vesicular and relatively symmetrical. There was only a scant cytoplasm in many of the cells, which was vesicular. Mitotic figures were not apparent. The size of the cystic cavities, the

absence of normal bronchial elements in the wall and the rela-
tively slight amount of inflammatory reaction present in the wall
all suggest that the lesion present here is one of congenital cystic
disease rather than that resulting from an acquired infection.

Case 3

W. T., male, age 39. At 3 years of age the patient had a suppurative
lymphadenitis and osteomyelitis which healed in approximately 3 years. At
9 years of age he developed pneumonia, following which he had a produc-
tive cough with foul sputum which had since been constant. There had
been no hemoptysis nor had there been any great change in his condition.
One year before his admission to the hospital the patient developed influ-
enza, at which time signs suggestive of pulmonary tuberculosis were found
and he was sent to a tuberculosis sanitarium. Here he was thought to have
bronchiectasis of the right lower lobe and was referred to the Barnes Hos-
pital where he was found to have large saccular dilatations chiefly confined
to the right lower lobe. On bronchoscopic examination these cavities were
found to be filled with pus. There was no evidence of obstruction to the
bronchi nor was there any evidence of pulmonary tuberculosis. Accordingly,
on August 27, 1940, the right lower lobe was removed by Dr. Brian Blades.
Because no evidence of an interlobar fissure was seen at the time of opera-
tion it was necessary to make a resection of that portion rather than to
perform an ordinary lobectomy.

Gross Pathology. Examination of the gross specimen showed
the entire surface to be covered by irregular patches of fibrous
tissue representing adhesions which had been divided at the time
of operation. Landmarks on the specimen were rather difficult
to identify. The posterior inferior portion of the lobe was dense
and of a firmer consistency than the anterior portion of the speci-
men. On cut section it was found that many of the larger bronchi
were markedly dilated. The walls of these bronchi were consid-
erably thickened. The entire posterior portion of the lobe was
almost completely consolidated and the anterior portion fairly
well aërated. In the posterior portion near the base there was a
large cyst measuring 1.5 by 2.5 cm.

Microscopic Pathology. The features of chief microscopical
interest were limited to the bronchi. The markedly dilated walls
showed considerable irregularity, there being many infoldings
with the production of an irregular lumen. The epithelium was
intact over a good portion of the bronchi. However, in certain
areas the mucosa was lost, leaving a connective tissue base. In
other areas the columnar epithelium lining the dilated bronchi

was thickened and there was a suggestion of a change to a squamous type. On the whole, however, there was a small amount of inflammatory exudate to be found in these dilated bronchi. There was almost complete absence of cartilage throughout the smaller bronchi and bronchioles. In several areas there was marked metaplasia of epithelial elements. These areas were often found in regions normally occupied by mucous glands and presented as large islands of cellular material, fairly well differentiated and showing only a few mitotic figures (Figs. 5 and 6). There was a striking tendency for growth along the subintimal portion of the pulmonary vein. Often the epithelium would project into the vein, giving the appearance of a thrombus of tumor tissue. In most instances, however, it was possible to see that the endothelium was intact overlying the metaplastic tissue. Adjacent to these areas of epithelial hyperplasia there could also be seen areas of new muscle formation without any appreciable relation to the normal architecture. This muscle was of the smooth type and was not associated with any bronchus. Examination of the lymph nodes showed chiefly sinus hyperplasia with epithelial elements. Other portions of the lung showed various phases of atelectasis and pneumonitis such as one would expect from the gross picture. Because of the obvious evidence of malformation presented in this lobe it was felt that the inflammatory process here was probably secondary and that the nature of the lesion was one of congenital cystic disease.

DISCUSSION

It is possible that objections might be raised to the consideration of these pathological processes as congenital cystic disease rather than as bronchiectasis. We have classified these lesions as congenital cystic disease because the lungs have shown evidence of developmental malformation. The fact that clinical symptoms in these three individuals did not develop until relatively late is of no significance. Frequently, clinical symptoms in congenital cystic disease of the lung do not occur until the development of an infectious process. Where cysts are not infected it is not unusual to find them symptomless.

The epithelial changes described in the three cases were not apparent to the naked eye. The blocks were examined for such

changes before sectioning. In no instance was there any evidence of extension to contiguous structures such as the mediastinum, nor was there any evidence of distant metastasis. These lesions were found only after careful search, requiring in some instances as many as fifteen separate blocks of tissue. While the process described obviously represents abnormal cellular growth, because of its local situation we have not felt justified in considering it malignant from a clinical standpoint.

While the changes mentioned do not resemble the picture seen in the so-called mixed tumor of the bronchus that we have previously described, we feel that they do represent a similar process; namely, a disturbance in the fundamental structural tissue growth so often seen in areas of abnormal tissue organization. Where this situation is encountered in other parts of the body it is not unusual to find malignant manifestations following environmental stresses and strains. Whether such abnormal epithelial proliferations as described here are concerned in carcinomatous change is a question that we shall consider in a subsequent publication.

CONCLUSIONS

Studies were undertaken to determine the presence of abnormal epithelial overgrowth in congenital malformation of the lung. In 3 of 9 patients operated upon for congenital cystic disease of the lung, evidence was found of such overgrowth which consisted for the most part of masses of poorly differentiated epithelial cells tending to appear as spindle cells or cuboidal cells showing a definite tendency toward invasion but nowhere presenting any evidence of metastasis. In none of these specimens was the lesion apparent to the naked eye.
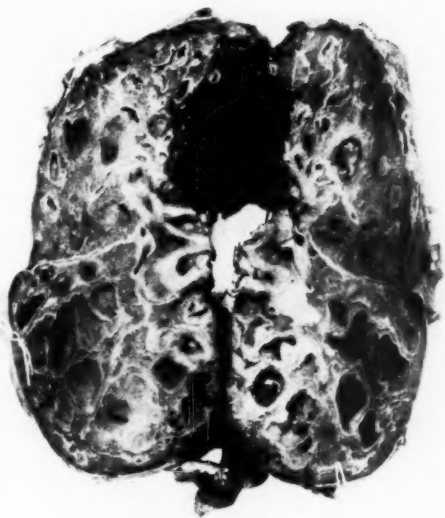
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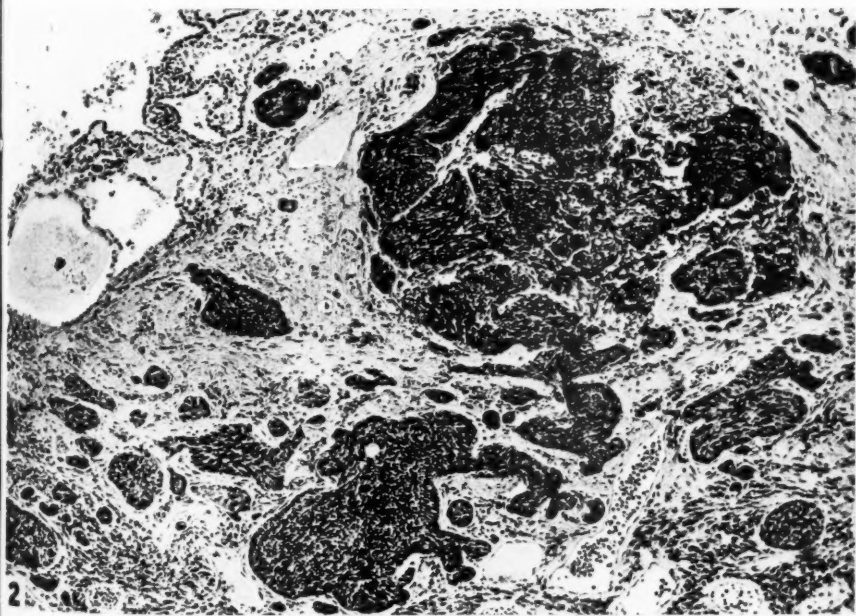
DESCRIPTION OF PLATES

PLATE 107

Fig. 1. Sagittal section through entire lung. The lower lobe presents numerous large cysts, the walls are moderately fibrosed and there is very little normal lung tissue present.

Fig. 2. An area of metaplastic epithelium in which the cells are for the most part spindle-shaped and the margins of the cellular areas are often clearly demarcated by palisading of the nuclei. There is no evidence of encapsulation. × 250.

FIG. 3.   A low power view of epithelial overgrowth showing relationship to the bronchial epithelium. There is a moderate round cell reaction throughout the wall of the bronchus. No cartilage is visible. The epithelium lining those air sacs adjacent to the bronchus is of cuboidal type. $\times$ 300.

FIG. 4.   Another area similar to that described in Figure 3 is shown. The epithelial masses give the appearance of having invaded lymph vessels. $\times$ 600.
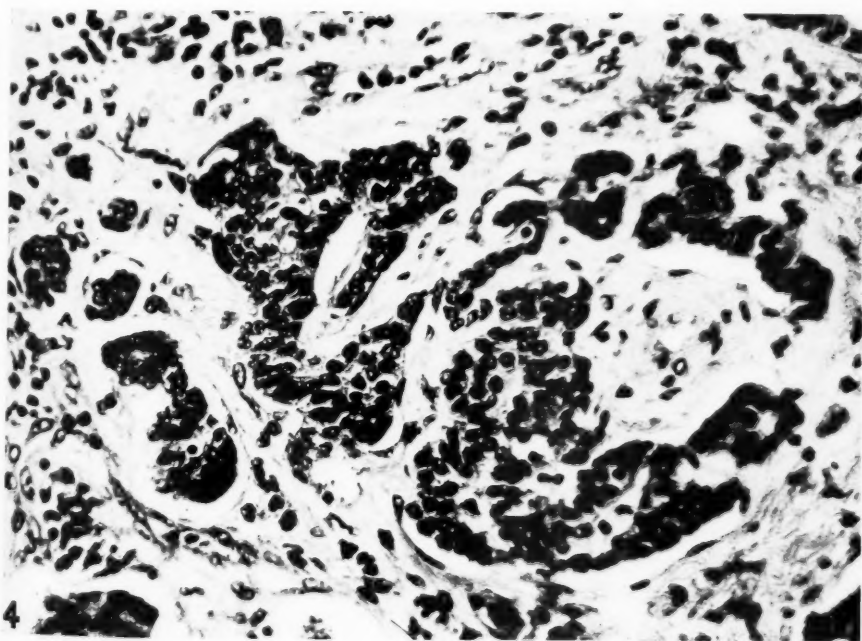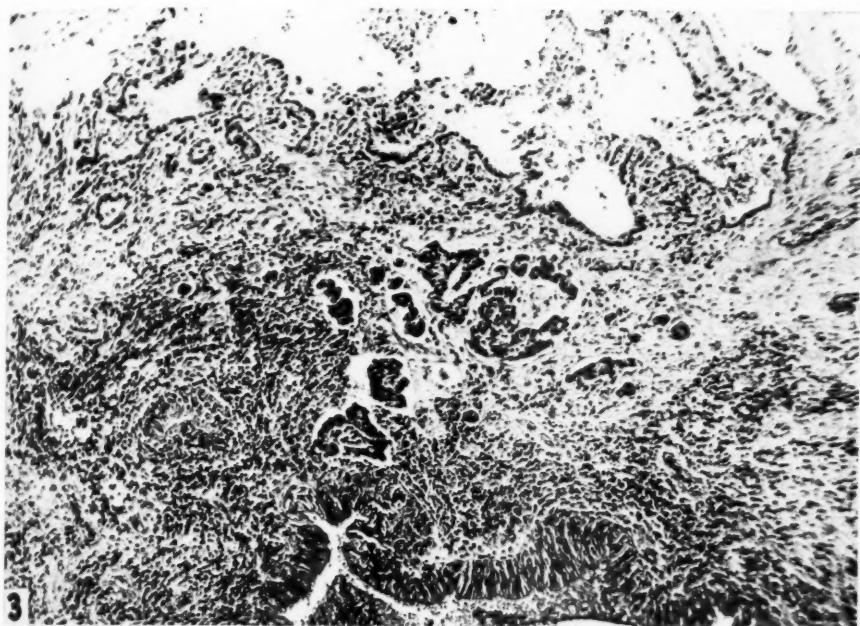
Womack and Graham   Metaplasia in Cystic Disease of Lung

FIG. 5. Low power view of an area of epithelial hyperplasia found well away from any bronchial wall. There are numerous small cavities and a large amount of smooth muscle is found in the stroma. These masses of cells are growing without any evidence of encapsulation. × 250.
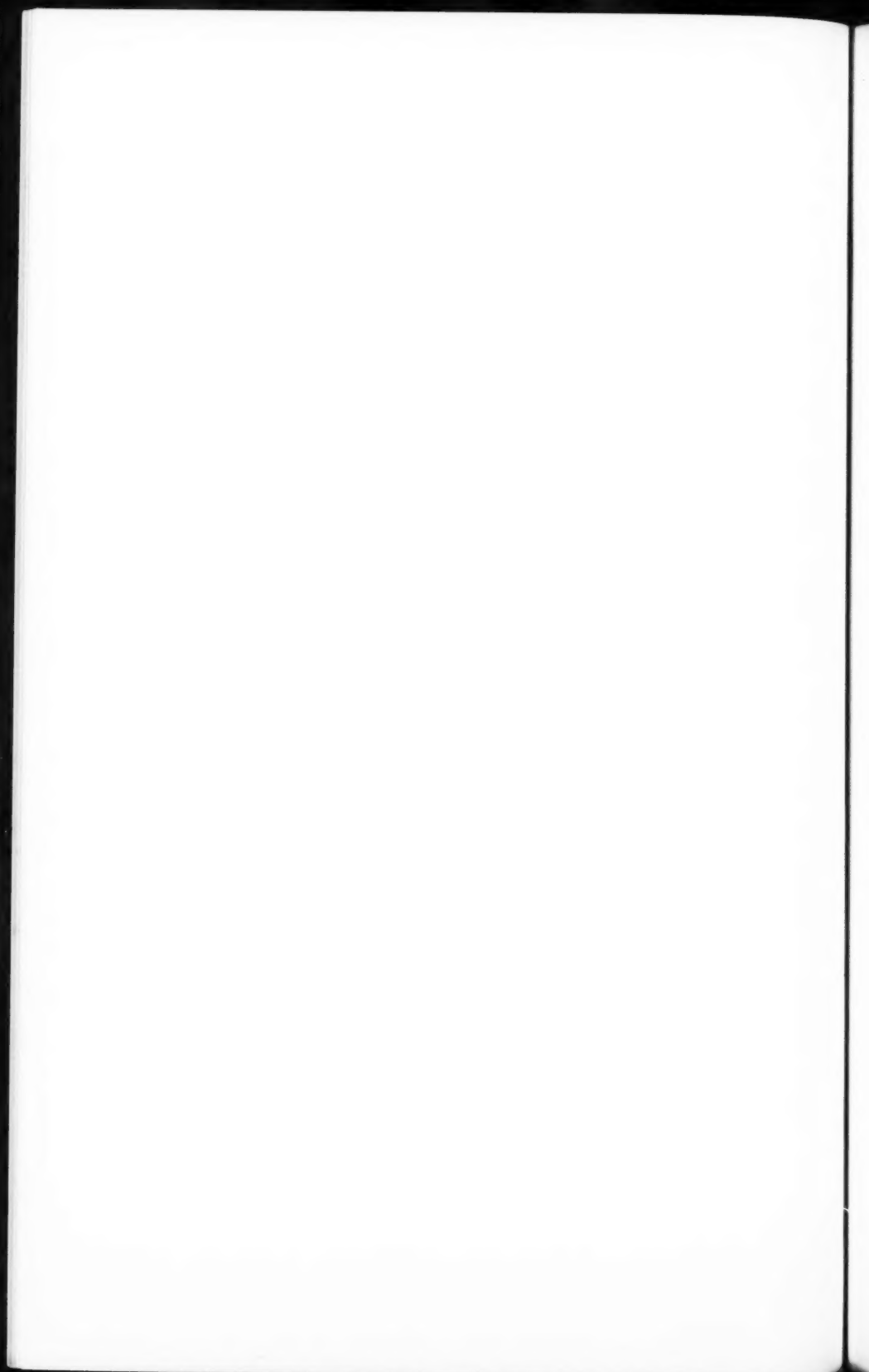
FIG. 6. Groups of epithelial masses segregated in a dense area of smooth muscle and fibroblasts. The cells tend to be spindle-shaped, although the margins of the masses evidence palisading of the nuclei. × 250.

Plate 109
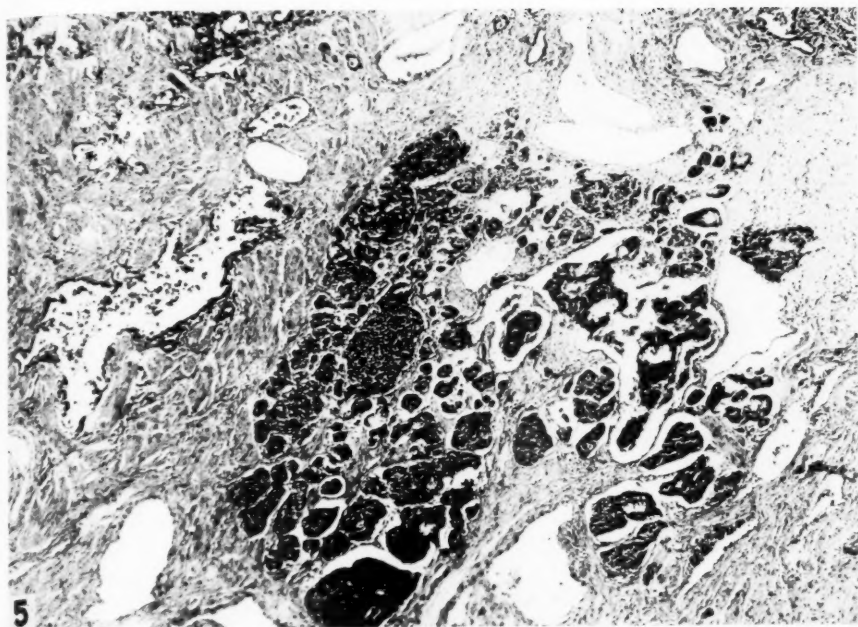
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HEREDITY AS DETERMINING THE TYPE AND SITE OF CANCER AND THE AGE AT WHICH IT OCCURS *

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I wish to present, tersely, evidence for the genetic influence in the occurrence of malignancy. Evidence will be advanced for the genetic control of: (1) the type of malignancy—carcinoma, sarcoma and leukemoid diseases; (2) the site of malignancy—breast, lung, body wall and subcutaneous tissues; (3) the age at which malignancy will occur.

The figures used are derived from the study of four strains of mice with their hybrid crosses. Tables I and II describe the material used in these studies. The total number of mice involved in the two series is 7332.

The parents selected from strain WQ for the reciprocal crosses shown in Table I were 3 litter sisters and their litter brother, derived from four inbred generations of breast carcinoma; that is, the mother, grandmother, great-grandmother and great-great-grandmother had each died of carcinoma of the breast.

Of the 3 litter sisters, ♀ I died of lung carcinoma at 16 months without breast carcinoma; ♀ II of hemorrhage at 6 months, too early to be tested for malignancy; ♀ III at 12 months of breast carcinoma. The litter brother died at 16.5 months of lung carcinoma. Therefore in strains 85/WQ and WQ/85 I, there are not only reciprocal crosses between the two strains of origin, but also reciprocal tests of lung carcinoma.

The parents chosen from strain 85 for the reciprocal crosses were 1 female and her 2 litter brothers, whose parents and grandparents died of intestinal infection without malignancy. The same male was used in crosses II and III. The female died at 11.7 months of intestinal infection, too early to be tested for malignancy, but her genetic behavior was closely like that of her litter brother I who died of multiple malignant liver adenomas at 24.5 months. The litter brother used for crosses II and III died of chronic nephritis at 19 months.

Table II shows series 2 and the ages and causes of death of the parents of the strains of origin 3 and 7, and of their two hybrid crosses.

TABLE I

Totals of Strains 85 and WQ and Their Reciprocal Crosses

Series 1		Total offspring	
WQ	10 inbred generations from ♀ (carcinoma, mammary gland, 15 mo.) X ♂ (chronic nephritis, 17 mo.)	♀ ♂	369 395 764
85	8 inbred generations from ♀ (pulmonary infection, 22.8 mo.) X ♂ (unknown infection, 6.4 mo.)	♀ ♂	423 502 925
Reciprocal crosses:			
85/WQ	8 inbred generations from ♀ (intestinal infection, 11.7 mo.) F_4 of 85 X ♂ (carcinoma, lung, 16.5 mo.) F_4 of WQ	♀ ♂	1133 1244 2377
WQ/85 I	8 inbred generations from ♀ (carcinoma, lung, 16.4 mo.) F_4 of WQ X ♂ (multiple liver adenomas, 24.5 mo.) F_4 of 85	♀ ♂	309 322 631
WQ/85 II	6 inbred generations from ♀ (retroperitoneal hemorrhage, 6 mo.) F_4 of WQ X ♂ (chronic nephritis, 19 mo.) F_4 of 85	♀ ♂	286 318 604
WQ/85 III	4 inbred generations from ♀ (carcinoma, mammary gland, 12 mo.) F_4 of WQ X ♂ (same as parent ♂ of II)	♀ ♂	122 121 243

TABLE II

Totals of Strains 3 and 7 and Two of Their Hybrid Crosses

Series 2		Total offspring	
Line 3	4 generations from ♀ (nephritis, 18.9 mo.) X ♂ (nephritis, 19 mo.)	♀ ♂	125 111 236
Line 7	9 generations from ♀ (carcinoma, mammary gland, 14.4 mo.) X ♂ (pneumonia, 10.9 mo.) Note: the strain was formed by a back-cross of the F_1 ♀ with the parent ♂	♀ ♂	402 365 767
Hybrid crosses:			
Line ¾	2 generations from F_2 ♀ of line 3 (hypertrophic heart, 19.6 mo.) X F_4 ♂ of line 7 (nephritis, 5.5 mo.)	♀ ♂	15 5 20
Line $\frac{7}{3/7}$	7 generations from F_5 ♀ of line 7 (intestinal infection, 12.5 mo.) X F_2 ♂ of line ¾ (sarcoma, body wall and subcutaneous tissues, 25.7 mo.)	♀ ♂	369 396 765

TABLE III

Percentage Incidence of Types of Malignancy in Series 1 and 2

Series 1	WQ	85	85/WQ	WQ/85 I, II, III	Totals reciprocal crosses
% carcinoma	92.3 (221)*	44.5 (236)	67.2 (944)	63.4 (568)	65.7 (1512)
% sarcoma	13.6 (22)	9.0 (210)	13.0 (690)	10.3 (369)	12.1 (1059)
% leukemia	64.0 (50)	22.2 (225)	25.4 (736)	40.8 (436)	31.1 (1172)

Series 2	3	7	¾	$\frac{7}{3/7}$	
% carcinoma	22.2 (81)	56.5 (418)	0.0 (20)	51.3 (318)	
% sarcoma	17.1 (82)	44.9 (390)	80.0 (20)	60.4 (386)	
% leukemia	20.9 (91)	17.4 (384)	0.0 (20)	21.8 (316)	

*The figures in parentheses represent the number of mice tested in each category.

Table III presents evidence for the genetic control of types of malignancy. The most rigid age test has been used in all of these figures. All noncancerous and cancerous mice dying before 22 months of age have been discarded as not adequately tested. Of course all mice actually showing the type and site of malignancy under consideration have been included, such mice obviously being tested for their type of malignancy at whatever age they died. Thus even very young cancerous mice with the tested type of malignancy are included, but noncancerous mice of the same and even greater ages are excluded as not certainly tested for cancer.

The average ages of the noncancerous mice in the different strains are from 25 to 29 months, and the age span of these mice is from 22 to 45.5 months. Mice in general reach their full development at 3 months or earlier. If 3 months in the life of a mouse is considered the equivalent of 12 years in the life of a human being, 12 months in a mouse would be the equivalent of 48 years, and 24 months would be the equivalent of 96 years of human life.

WQ is a strain in which only 5.6 per cent of the total offspring lived beyond 22 months. Over 78 per cent of the strain died at an average age of 13 months, the females nearly all of breast carcinoma, and the males of nephritis and wounds. The strain

is therefore inadequate for testing the genetic potentiality of any form of malignancy except breast carcinoma, since the mice do not live long enough to show whether or not they are susceptible to malignancy of any other site.

Examining the percentages for types in Table III, let us first consider carcinoma. Strain WQ had 92.3 per cent of carcinoma, most of which was carcinoma of the breast, in 221 tested mice. Strain 85 had 44.5 per cent of carcinoma (a low percentage of which was in the breast) in 236 tested mice. The reciprocal crosses showed 67.2 per cent where the male came from the strain having a high percentage of carcinoma; and 63.4 per cent where the female came from the strain having a high percentage of carcinoma. Both of these figures are nearer to the percentage for strain 85 than to that for strain WQ, but they show that where a high percentage of carcinoma was bred in, a high percentage of carcinoma came out in the resulting strain; and that when a strain having a high percentage of carcinoma was crossed with a strain having a lower percentage of carcinoma, both were effective. The average result, where the number of tested mice is large enough, shows a figure between the high and the lower percentages, and nearer to the lower.

Of sarcoma, WQ had only 13.6 per cent. Only 22 mice, however, lived into the test age for sarcoma. Strain 85 was adequately tested for sarcoma. Of 210 mice living well into the test age, only 9 per cent showed sarcoma. The resulting reciprocal strains both had a low percentage of sarcoma. As the percentages show, many of these mice had more than one type of malignancy. Many showed two types and some all three types developing concurrently.

Of leukemic disease, WQ had 64 per cent among the 50 mice that lived into the test age. Strain 85 had 22.2 per cent among 225 tested mice. The reciprocal hybrid strains showed percentages between those for WQ and 85 and nearer to the lower percentage strain.

Series 2 has been selected because of the contrast it offers to series 1. In this series, strain 3 showed a medium amount of malignancy of each type, and strain 7 showed a high percentage of carcinoma and of sarcoma, 56.5 and 44.9 per cent respectively, and only 17.4 per cent of leukemia.

Of the hybrid derivatives from these lines, 3/7 was a small strain of only 20 mice, 15 females and 5 males. All of the females and 1 of the males had sarcoma. The 4 males that did not develop any form of malignancy were mice that fought and died of wounds and extreme emaciation. All, however, were age-tested for every form of malignancy. Small as the strain is, and although perhaps it does not show its full malignant potentiality, it is striking that among 20 mice a sarcoma strain of 80 per cent was secured in which all of the malignant mice showed only one type of malignancy. It suggests an extracted line of sarcoma.

That it was primarily a sarcoma strain is shown by the results of its further hybridization. When a male from this strain, with sarcoma, was crossed with a female from strain 7 which was high in both carcinoma and sarcoma, to produce strain $\frac{7}{3/7}$, the first generation hybrids showed no carcinoma but only sarcoma. Carcinoma came out in the later generations. The totals for the hybrid line gave 60.4 per cent of sarcoma, a percentage lying between those of the sarcoma lines of higher and lower percentages. Again the influence of the high carcinoma line is shown

TABLE IV

A Realignment of the Percentages Given in Table III

	Approximately 100%	1 out of 2	1 out of 4	1 out of 8	0%
Carcinoma	92.3 (221)*	67.2 (944)	22.2 (81)		0 (20)
		63.4 (568)			
		56.5 (418)			
		51.3 (318)			
		44.5 (236)			
		60.3 (2484)			
Sarcoma	80.0 (20)	60.4 (386)		17.1 (82)	
		44.9 (390)		13.6 (22)	
				13.0 (690)	
				10.3 (369)	
				9.0 (210)	
		52.6 (776)		11.9 (1373)	
Leukemic disease		64.0 (50)	25.4 (736)	17.4 (384)	0 (20)
		40.8 (436)	22.2 (225)		
			21.8 (316)		
			20.9 (91)		
		43.2 (486)	23.7 (1368)		

* The numbers in parentheses represent the number of tested mice.

in the totals of this hybrid cross which gave 51.3 per cent of carcinoma, slightly less than the 56.5 per cent of strain 7.

From these strains there were developed (Table IV):

1. Approximately 100 per cent strains of carcinoma and of sarcoma.

2. High percentage strains (about 1 out of 2) for each type.

3. Medium percentage strains (about 1 out of 4) for carcinoma and leukemic disease.

4. Low percentage strains (about 1 out of 8 or more) for sarcoma and leukemic disease.

5. One 0 per cent strain for carcinoma and for leukemia.

These facts demonstrate the genetic control of susceptibility to types of malignancy, irrespective of sites. They show that when sarcoma or leukemia is bred in, sarcoma or leukemia comes out in the resulting lines; that when carcinoma is bred in, carcinoma comes out in the strain; and that when all three types are bred in, it is possible to extract lines some of which show all three

TABLE V

Incidence of Breast Carcinoma and Ovarian Adenoma in Series 1 and 2

Series 1	% mammary gland carcinoma	% ovarian adenoma
WQ	95.1 (203)*	11.1 (36)
85	14.7 (163)	30.3 (165)
Reciprocal crosses:		
85/WQ	38.1 (1 out of 3−) (698)	37.5 (552)
WQ/85	46.5 (1 out of 2+) (387)	32.4 (259)
Total of reciprocal crosses:	41.1 (1085)	35.9 (811)
Series 2		
3	0 (124)	2.1 (48)
7	0 (402)	48.2 (247)
3/7	0 (15)	0 (15)
$\frac{7}{3/7}$	0 (369)	42.0 (169)
Totals:	0 (910)	

* The numbers in parentheses represent the number of tested mice.

types of malignancy, some of which show two types of malig-
nancy, and some of which show one type and only one type.

As evidence for the genetic control of the site of malignancy,
I have chosen four tumor sites appearing in these two series:
carcinoma of the mammary gland, of the ovary and of the lung,
and spindle cell sarcoma of the body wall and subcutaneous
tissues.

In series 1, Table V, WQ showed 95.1 per cent of breast carci-
noma among 203 age-tested females, and the parent female of
the strain had breast carcinoma. Strain 85 showed only 14.7 per
cent of breast carcinoma (about 1 out of 7) among 163 age-tested
females.

In the reciprocal crosses: 85/WQ, in which the parent female
came from the strain with low breast cancer, showed 38.1 per
cent of breast cancer in 698 tested females (1 out of 2.6);
WQ/85 I, II, III, in which the parent females came from the
strain with high breast cancer, showed 46.5 per cent of breast
cancer in 387 tested females (1 out of 2.2). There is no evidence
here of any extrachromosomal factor, nor any evidence for domi-
nance of breast location. On the contrary, the figures show a
closer approximation of the hybrid strains to the strain with a
low percentage of cancer of the mammary gland, whether the
male or the female is from the strain with a low percentage of
breast cancer.

Series 2 shows an interesting sequence of strains from which
breast cancer was entirely eliminated, as there was none in strains
3 or 7 or in either of their hybrid derivatives, this series totaling
910 age-tested females. Since breast cancer is the common malig-
nancy reported in mice, this is strong verification of a genetic
influence in the occurrence of breast-location for malignancy,
and of the possibility of breeding it out of families. It is also
evidence for the existence of separate factors for the type of
carcinoma and its site incidence, for in series 2 there was a high
incidence of carcinoma, but no breast-location of the malignancy.

In the findings for lung carcinoma (Table VI) in series 1,
there is no evidence for an extrachromosomal factor, wherein they
are in agreement with the findings of other workers. Neither is
there evidence for any sort of a dominant, nor for either sort of
unit character. Rather, in the figures for lung carcinoma there

TABLE VI

Incidence of Lung Carcinoma in Series 1 and 2

	% lung carcinoma	No. tested
Series 1:		
WQ	No estimate	19
85	7.4	204
Reciprocal lung crosses:		
85/WQ and WQ/85 I	24.7	943
Crosses from parents without lung carcinoma:		
WQ/85 II and III	12.4	178
Series 2:		
3	19.8	81
$\frac{7}{7}$	32.9	398
$\frac{7}{7}$	0.0	20
$\frac{7}{3/7}$	31.3	307

is evidence for a minimum of two recessive factors, one for type and one for site.

The incidence of body wall and subcutaneous spindle cell sarcoma (Table VII) was noticeably different in series 1 and 2. In series 1, the percentages were consistently low in the strains of origin and in the reciprocal crosses. In series 2, the percentages were high.

From the strains here reported there were secured:

1. One approximately 100 per cent strain for breast location, and one for body wall and subcutaneous locations.

2. Strains of high percentage (about 1 out of 2) for breast, ovary and body wall locations.

TABLE VII

Incidence of Body Wall and Subcutaneous Sarcomas in Series 1 and 2

	% sarcoma	No. tested
Series 1:		
WQ	9.5	21
85	6.7	208
Reciprocal crosses:		
85/WQ	10.4	681
WQ/85	7.9	367
Series 2:		
3	17.1	82
$\frac{7}{7}$	42.9	387
$\frac{7}{7}$	80.0	20
$\frac{7}{3/7}$	56.2	377

3. No strains of high percentage for lung location, but strains with about 1 out of 4 for lung location.

4. Strains with about 1 out of 8 or more for breast, ovary, lung and body wall locations.

5. Strains with 0 per cent of breast, lung and ovary locations. These are not ratios for dominance.

TABLE VIII

Deaths from All Causes in Entire Strains, Giving the Percentage of Totals Living into Late, Median and Early Age Spans

Series 1	No. mice	% late	% median	% early
WQ	338	5.6	16.0	78.4
85	449	45.0	20.7	34.3
Reciprocal crosses	2143	47.5	20.3	32.2

Late = over 22 months; median = 19 to 21 months; early = under 18 months.

TABLE IX

Average Age of Malignancy in the Original and Hybrid Strains with the Percentage Distribution of the Tumors in Late, Median and Early Age Groups

Series 1	No. cancerous mice	Average age in months	Age span of malignancy in months	% late	% median	% early
WQ	222	14.0	4.4—26.2	1.3	12.2	86.5
85	157	22.8	8.9—32.4	59.9	19.1	21.0
Reciprocal crosses:						
F₁	26	24.3	12.4—35.3	57.7	11.5	30.8
Totals	1249	22.2	3.1—38.2	49.9	16.0	34.1

Late = over 22 months; median = 19 to 21 months; early = under 18 months.

Table VIII gives a consideration of deaths from all causes in series 1, showing the percentage of distribution of deaths in age periods: late (over 22 months); median (from 19 to 21 months) and early (under 18 months). Table IX gives the percentage of incidence of the cancers in the three age spans.

WQ was a short-lived family: only 5.6 per cent lived to be over 22 months old, and 78.4 per cent died at an average age of 13 months. These figures include death from all causes, non-malignant and malignant. Whatever the cause of death of these mice might be, they died early. Strain 85 was a long-lived strain: 45 per cent lived from 22 to 34 months, and 34.3 per cent died under 18 months, this group including 46.6 per cent of the fighting males. The reciprocals derived from the crosses between the

long-lived and the short-lived strains were long-lived: 47.5 per cent died from 22 to 45.5 months, and 32.2 per cent died under 18 months.

As to the age-incidence of malignancy, strain WQ had an average age at death of 14 months. Only 1.3 per cent of the mice with malignant neoplasms died after 22 months, whereas 86.5 per cent died at an average age of 13 months. In strain 85, the reverse was true. Nearly 60 per cent of the mice with malignant neoplasms died between 22 to 32.4 months of age and only 21 per cent under 18 months. The average age for malignancy was 22.8 months. In the reciprocal crosses, nearly 50 per cent died between 22 to 38 months of age and 34.1 per cent under 18 months. The average age for malignancy was 22.2 months. The age span of those with malignancy was 3.1 to 38.2 months.

TABLE X

Comparison of the Age Incidence of Tumors of the Same Type in the Original and Hybrid Strains

Series 1		Average age in months	Age span of malignancy in months	% late	% median	% early
	No. mammary gland tumors					
WQ	193	13.2	6.9—21	0	10.4	89.6
85	24	21.1	13.2—28.1	50.0	12.5	37.5
Reciprocal crosses	446	18.2	6.2—32.5	23.6	15.9	60.5
	No. lung carcinomas					
WQ	9	16.5	10.4—24	11.1	22.2	66.7
85	15	25.9	17.2—32.4	86.7	0	13.3
Reciprocal crosses	255	22.9	9.3—36.3	59.6	18.8	21.6
	No. leukemic disease					
WQ	32	14.5	4.4—23.1	3.1	18.8	78.1
85	50	22.1	11.6—31	52.0	26.0	22.0
Reciprocal crosses	365	22.5	3.1—37.4	57.8	18.6	23.6

Late = over 22 months; median = 19 to 21 months; early = under 18 months.

These same facts held regardless of the type or site of malignancy. In the strains with late malignancy, even breast carcinoma, ordinarily an early tumor, occurred as late as 32 months. In the strains with early malignancy, such usually late tumors as lung and liver malignancy occurred as early as 8 months. The average age for leukemia in the strains with early malignancy

was 14 months, and in the strains with late malignancy, 22.5 months.

In age-incidence for malignancy in general and in the ages for specific types and sites of malignancy, the hybrids uniformly resemble more closely the long-lived strain. In the age at death from all other causes also, the hybrids more closely resemble the long-lived strain. Thus the tendency for death from all causes to be late within a strain seems dominant over the tendency to die early. The tendency to have malignancy late seems to be dominant over the tendency to have malignancy early.

What is the significance of late and early malignancy and its genetic control? May it mean the hereditary transmission of greater and of less resistance to the causative factors involved in the mutation, whether these causative factors are cancerogenic chemicals, hormones, a virus or any other form of chronic irritant? Or does it mean that genetic constitution determines the timespan within which tissues and organs are capable of normal function with regard to all diseases? May it mean that genetics can control the production of an organism with a high degree of resistance against all disease attacks, malignant or nonmalignant, or an organism which will succumb early to any assault?

In any event the tendency to late malignancy is dominant over the tendency to early malignancy, just as the tendency to live long is dominant over the tendency to die early. The dominance of greater resistance over less resistance is demonstrated, which is consistent with the theory of the recessive nature of cancer susceptibility.
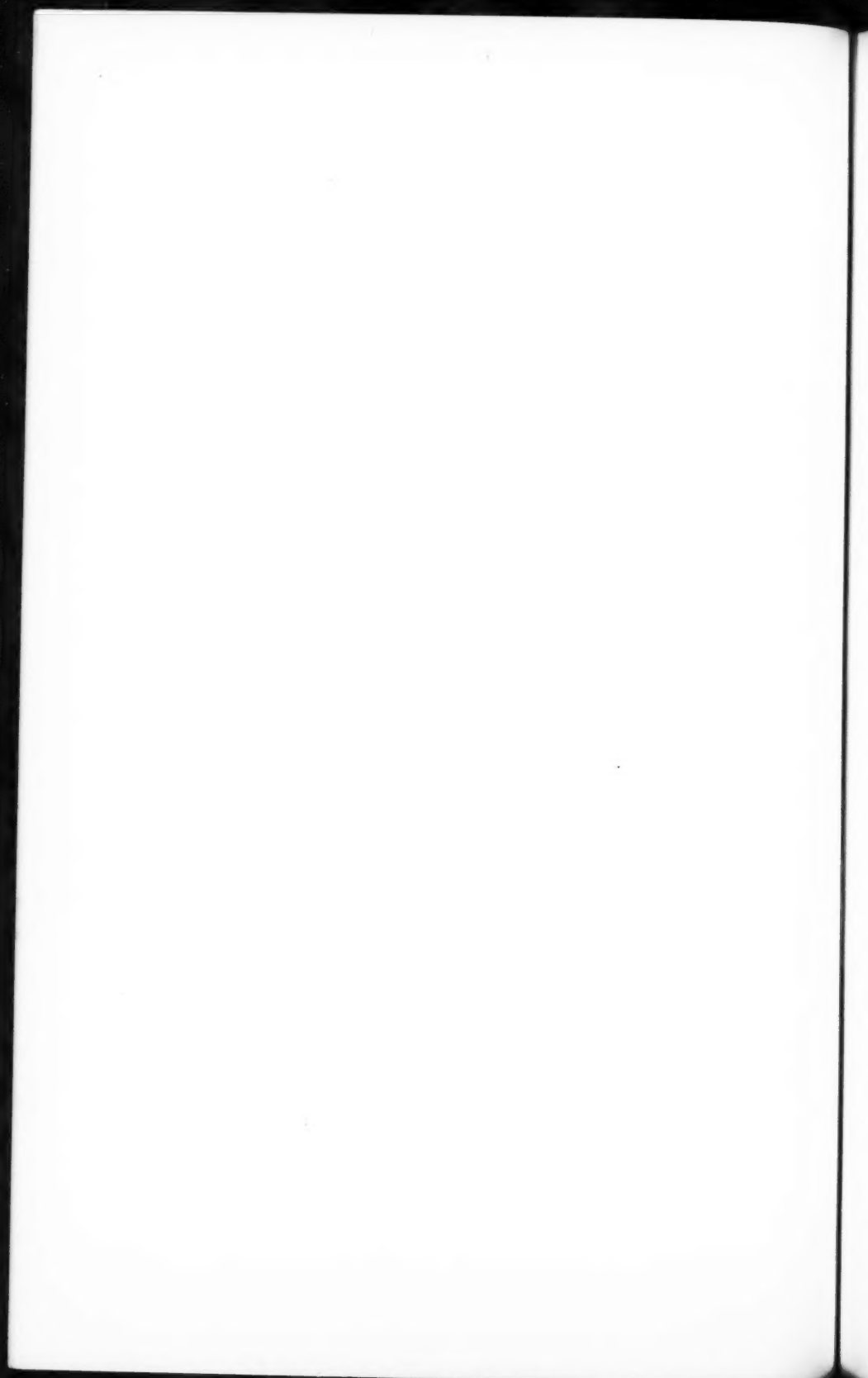
THE NONSAPONIFIABLE LIPID FRACTION OF LIVERS FROM CANCEROUS AND NONCANCEROUS PERSONS [*]

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In a recent communication [1] the induction of spindle cell sarcomas in mice by the subcutaneous injection of the nonsaponifiable lipid fraction of livers from persons having cancer was described. The purpose of this paper is to describe such extracts in greater detail, giving the amounts which human livers contain and attempting to make some preliminary correlations of this fraction with the nature of the major disease and with the morphology of the liver.

The original nonsaponifiable lipid extract which proved to be cancerogenic was prepared from the pooled livers of 8 persons. From 9,420 gm. of fresh liver the yield was 65.6 gm. Since that time individual extractions have been made of livers from 33 persons having various kinds of tumors, the livers themselves containing cancer only to the degree stated in Table I. The livers of 19 adult persons not having cancer were similarly extracted. Most of these individual extracts are now under test in animals for cancer-producing ability.

METHOD OF CHEMICAL EXTRACTION

The livers were ground as soon after autopsy as possible and were preserved in an equal volume of 95 per cent alcohol. They were saponified for about 18 to 24 hours by alcoholic potassium hydroxide on a steam bath under a reflex condenser, water being added in a volume equal to that of the alcohol. The amount of potassium hydroxide used was 10 gm. per 100 gm. of liver tissue. The material was then extracted four times with ethylene dichloride. These combined extracts were dehydrated with anhydrous sodium sulfate, filtered, and then evaporated to dryness at reduced pressure. The residue was resaponified for 4 hours with alcoholic potassium hydroxide, without water. For this saponification 0.5 gm. of potassium hydroxide was used for each 100

TABLE I

Nonsaponifiable Lipids of Livers from Persons Having Neoplasms

No.	Major diagnosis	Liver weight in gm.	% non-sapon-ifiable lipids	Total non-saponifi-able lipids in gm. (calcu-lated)	Fatty changes (histological)	Miscellaneous
H-40-191	Carcinoma, tongue	1200	1.28	15.3	Moderate	
4961	Carcinoma, esophagus	1735	0.44	7.6	Slight	Also shows moderate portal cirrhosis
4968	Carcinoma, esophagus	1700	0.55	9.3	Slight	Also shows a slight portal cirrhosis
5016	Carcinoma, stomach	1600	0.38	6.0	None	
5037	Carcinoma, stomach	2010	0.82	16.5	Slight	
5039	Carcinoma, stomach	1800	2.78	41.2	Slight	
4984	Carcinoma, colon	?	0.63	?	Very slight	
4990	Carcinoma, colon	1200	0.65	8.8	Slight	Occasional microscopic metastasis
4938	Carcinoma, lung	1900	0.69	13.2	Slight	
4948	Carcinoma, lung	1350	0.56	7.6	None	
H-40-186	Carcinoma, lung	2250	0.64	10.3	Slight	
H-40-183	Carcinoma, prostate	2150	1.23	26.5	Slight	Liver also shows cir-rhosis; contains 3 cav-ernous hemangiomas
H-40-178	Carcinoma, prostate	1900	0.64	12.2	Slight	
5022	Carcinoma, urinary bladder	1550	0.51	7.9	Slight	
4958	Carcinoma, breast	910	0.70	6.4	Very slight	
4966	Carcinoma, breast	1640	0.40	6.5	Slight	
4991	Carcinoma, breast	1250	0.81	10.1	Slight	Occasional small metastases
4927	Carcinoma, ovary	1960	1.15	22.5	Slight	Occasional tiny metastases
4992	Carcinoma, ovary	1800	0.95	17.2	Severe	
CCH-4-145	Carcinoma, ovary	1900	0.45	8.5		
CCH-40-485	Carcinoma, adrenal	2980	0.53	15.8	Slight	Estimated 10% metastases
H-40-192	Retroperitoneal sarcoma	1790	0.83	14.9	Moderate	
4943	Lymphoblastoma	1550	0.57	8.8	Moderate	No infiltration into liver
H-40-193	Lymphosarcoma	1560	2.33	36.2	Slight	No infiltration into liver
4974	Reticulum cell sarcoma	1070	0.34	3.6	Slight	No infiltration into liver
5012	Reticulum cell sarcoma	1500	0.32	5.2	Very slight	Small infiltrations into liver
5048	Reticulum cell sarcoma	1350	0.74	10.0	Slight	Small infiltrations into liver
5014	Monocytic leukemia	2050	0.46	9.5	Very slight	Estimated 15-20% leukemia
H-40-231	Hodgkin's disease	1730	0.45	8.2	Slight	Slight periportal infiltration
5013	Meningioma	1450	0.41	6.0	Moderate	
4946	Chromophobe adenoma of the pituitary	1775	0.63	11.1	Very slight	
4980	Carcinoma, branchial cyst	1500	0.33	4.9	Very slight	
H-40-180	Carcinoma, parotid	1230	0.55	6.7	Moderate	Also shows advanced cirrhosis
Average		1665	0.75	12.3		

gm. of liver, original weight. Extraction with ethylene dichloride was again carried out for four times and the pooled extracts were evaporated to dryness.

The final residue was a flaky, yellow to orange-brown solid with a pungent, penetrating, disagreeable odor.

As given in Tables I, II and III the total nonsaponifiable lipids for each liver were calculated from the percentage of nonsaponifiable lipids and the known total weight of the organ. This was frequently greater than the amount actually extracted because all of the liver was not available for chemical study in most cases.

TABLE II

Nonsaponifiable Lipids of Livers from Persons Not Having Cancer

No.	Major diagnosis	Liver weight in gm.	% non-saponifiable lipids	Total non-saponifiable lipids in gm. (calculated)	Fatty changes (histological)
4945	Lobar pneumonia	1650	0.50	8.2	None
4964	Lobar pneumonia	2080	0.38	7.9	Slight
4977	Ulcerative colitis, nonspecific	1790	0.41	7.3	None
4985	Ulcerative colitis, nonspecific	2200	0.64	14.0	Severe
4940	Abruptio placenta	2560	0.73	18.7	Moderate
4965	Bacterial endocarditis	2200	0.69	15.2	Slight
4971	Syphilitic aortitis	1570	0.64	10.1	Moderate
4973	Polycystic kidneys	1775	0.37	6.5	Slight
4975	Pneumococcic meningitis	2530	0.71	17.9	Slight
4979	Hypertensive heart disease; uremia	1480	0.26	3.9	Moderate
Cor.-18–5–40	Lye poisoning	1710	0.84	14.4	Slight
Average		1959	0.56	11.3	

TABLE III

Nonsaponifiable Lipids of Cirrhotic Livers

No.	Type of cirrhosis	Stage of cirrhosis (microscopical)	Liver weight in gm.	% non-saponifiable lipids	Total non-saponifiable lipids in gm. (calculated)	Fatty changes (histological)
4951	Portal	Advanced	2400	0.76	18.2	Slight
4960	Biliary	Early	1500	0.72	10.7	Slight
Cor.-20–5–40	Portal	Advanced	1980	0.66	13.0	Severe
Cor.-65–5–40	Portal (?)	Mild, inactive	1415	0.94	13.3	Slight
CCH 40–546	Portal	Advanced	1650	0.33	5.4	Severe
Cor.-37–6–40	Portal	Moderate	1340	0.76	10.2	Slight
Cor.-49–6–40	Portal	Moderate	1340	1.47	19.7	Slight
5063	Cardiac	Early	1250	0.64	8.0	Moderate
Average			1609	0.78	12.3	

RESULTS

A study of Table I shows that the nonsaponifiable lipid fraction of livers in persons with cancer varied from 0.32 per cent to 2.78 per cent (moist weight). The total nonsaponifiable lipid fraction obtained from human livers weighed between 3.6 and 41.2 gm. The individual variation was enormous. There did not seem to be any correlation between the type of neoplasm and the amount of nonsaponifiable lipids. Neither did there appear to be a significant change in the nonsaponifiable lipids if the primary tumor was located in the region of portal blood drainage.

In Table II the results of similar studies on the livers of persons not having cancer are recorded. These also showed great variation in the amount of nonsaponifiable lipids from case to case, although somewhat less so than in patients with cancer. Although the percentage of nonsaponifiable lipids averaged less for these livers, the total weight of this fraction was just as great as in Table I because the average weight of these livers was greater. This appeared to be true because the average age of these patients was less and therefore there was less brown atrophy of the liver. Also these patients did not show cancer cachexia.

The livers showing cirrhosis were analyzed separately because cirrhosis of the liver may be considered a potentially cancerous or in a sense even a precancerous lesion.[2] The same great individual variation in the nonsaponifiable lipid fraction was shown as in the other diseases, and although the total amount was greater than was shown in the group without cancer, this is probably not significant. In the cases showing cirrhosis there did not appear to be any relationship between the type or the severity of the cirrhosis and the yield of nonsaponifiable lipids. The results are shown in Table III.

In each of the three tables is given the amount of fatty change visible in the livers by microscopical examination of frozen sections stained by scarlet red, and quantitatively expressed crudely as none, slight, moderate or severe. These fatty changes included both fatty degeneration and fatty infiltration and no attempt was made to distinguish between them. There was no correlation between the amount of fatty change visible on microscopical examination and the amount of nonsaponifiable lipids extracted by this method.

It is possible that quantitative or qualitative differences between cancerous and noncancerous persons may be discovered when the nonsaponifiable lipid extracts are fractionated.

Summary

The total nonsaponifiable lipids recovered from the livers of 33 persons with cancer, 11 with various non-neoplastic diseases and 8 with cirrhosis of the liver, show no significant differences in amount. The individual difference is enormous and is not correlated with the type of tumor, with the location of the primary tumor, or with the amount of fatty change (fatty degeneration and fatty infiltration) visible in microscopical sections.

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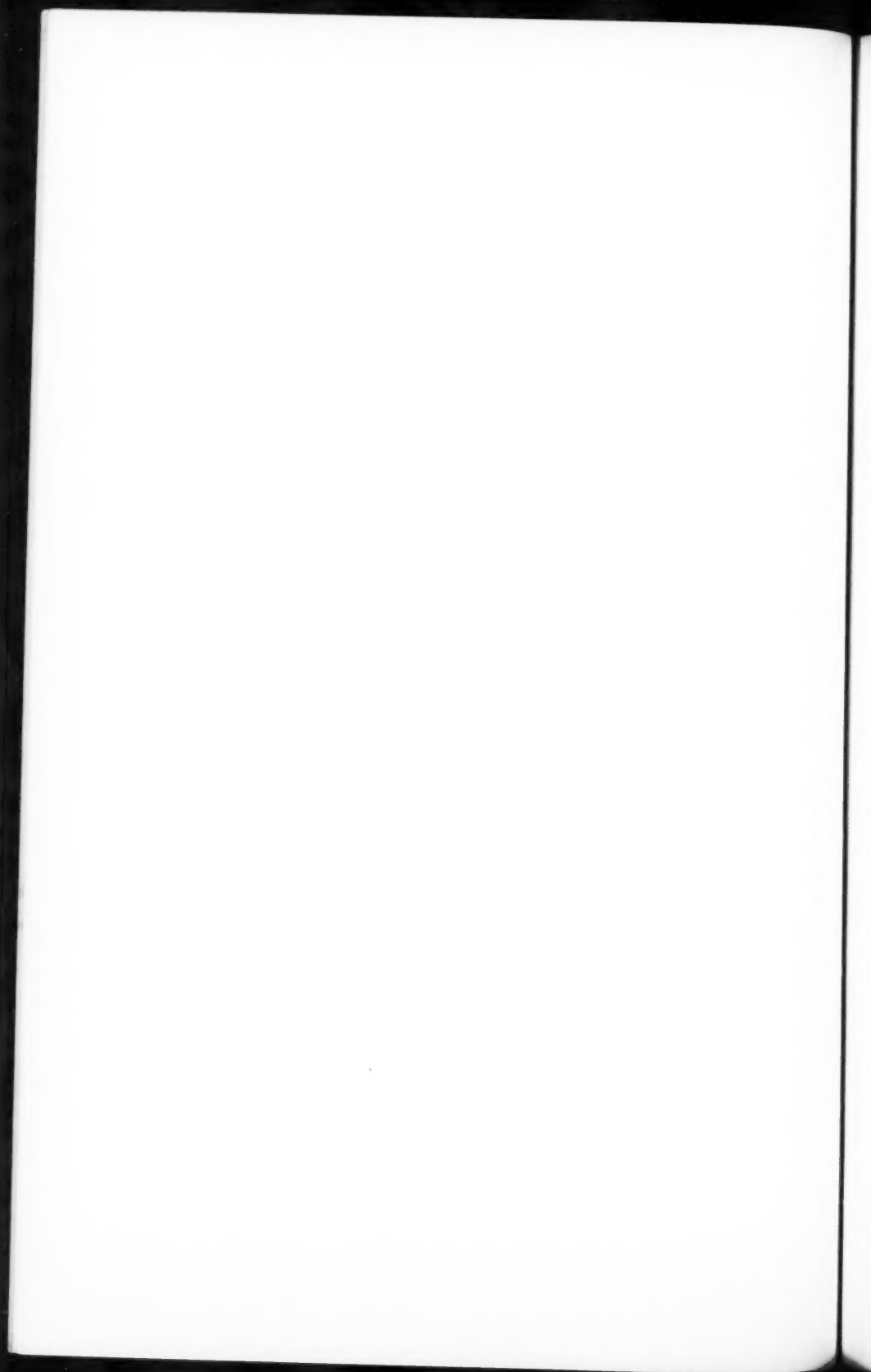
VARIATION IN THE COMPOSITION OF GALLSTONES SIMULTANEOUSLY FORMED IN THE GALLBLADDER*

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During a period of simultaneous formation of gallstones there is usually considerable freedom of movement of the stones within the gallbladder, and the bile in which they are bathed at any given time is usually of about the same chemical composition throughout the gallbladder. Under these conditions the building material laid down at any one time is of about the same composition in one stone as in another and since the physical conditions of deposition and crystallization are likewise similar throughout, the stones also tend to be similar in form and size. However, with variation in the composition of the bile, in the freedom of its flow and in the pathological state of the gallbladder wall during the period of stone formation, there may be equally great variation in the composition of the different layers of each of the similar stones. This accounts also in large measure for the marked difference in aggregations or crops of gallstones formed in different individuals and even in the same individual at different times. Also any deposits which may be laid down on preëxisting single or multiple stones during a period of formation of an aggregation of stones are usually of the same general composition as the newly formed stones.

Cholesterol stones are frequently seen in small aggregations ranging up to six or seven in number, in which case they grow to be of large size. Up to a certain point they remain mobile, are all bathed in approximately the same biliary content and are very similar in composition. But on exceeding a certain size, they form a chain within the gallbladder, become faceted where their ends come in contact, and partially obstruct the lumen. This obstruction results in stagnation of the bile which increases in degree in each compartment proceeding from the ampullar region toward the fundus. If there is further growth of the stones, the difference in composition of the bile and in degree of stagnation in each

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compartment may cause a difference in composition of the new deposit on each stone. The authors and Pepinsky [1] have reported cases in which there was an increasing tendency for the deposition of calcium and pigment on such stones proceeding from ampulla to fundus of the gallbladder. This is in line with the observation reported in the same article that there is an increasing tendency for the deposition of calcium and pigment on stones formed in the presence of increasing degree of obstruction of the cystic duct, either from stone or from inflammation.

Further observations have been made on the subject. Aronson [2] has reported that much of the dark discoloration of some gall-stones is due to a substance which does not give the tests for bile pigments but, judging from stoichiometric analysis, probably consists of polymers containing pyrrole derivatives which are products of degradation of the bile pigments. While in some cases the more recent deposits on the stones contain increasingly more calcium and are increasingly darker in color from ampulla to fundus, in others no calcium is present but they are increasingly darker from ampulla to fundus. These differences in composition occur in varying degrees of intensity in a considerable percentage of the cases in which a chain of large gallstones fills the greater portion of the gallbladder, and the more nearly it is filled the more frequent the occurrence. Some of the features of this variation in composition of stones formed simultaneously are illustrated in the following cases.

REPORTS OF CASES

Case 1

A man, 54 years old, died of abdominal sarcomatosis after an illness of 6 months characterized by weight loss and attacks of abdominal pain. One month before death an oval, firm mass was palpable in the gallbladder region and a roentgenogram of that region revealed two large ring-shaped, radio-opaque shadows, the distal shadow being more opaque than the proximal (Fig. 1). Attempted cholecystography resulted in nonvisualization of the gallbladder by the dye.

At autopsy the *gallbladder* measured about 12 cm. in length and was filled with a solid mass of stones. Its wall was thickened and there was sarcomatous implantation on much of its serous coat. On opening it there were found four large articulated stones filling nearly all of the lumen and thirteen small faceted stones

situated about a large stone in the fundus. About 15 cc. of greenish yellow, turbid fluid was present. The cystic duct was patent but the ampulla was blocked and the outlet of the gallbladder was partly obstructed by the large proximal stone (Fig. 2). On inspection it was seen that the large fundal stone was faceted proximally where it contacted the adjacent large stone and on two sides from contact with the smaller stones. The stones were variously colored by bile pigments and in general they were increasingly dark from the ampulla to the fundus. Roentgenograms were made of the intact stones and of slices cut from the center of each large stone (Fig. 3). The proximal stone showed no calcium shadow. The second stone showed lamina of extremely faint calcium density near the periphery. The third stone showed dense calcium shadows on one side and fainter shadows on the opposite side and along the articulated surfaces. The large fundal stone showed a heavy calcium shadow about the entire periphery and a fainter ring of calcium internal to this. The thirteen small fundal stones showed faint central, and heavy circular peripheral, shadows of calcium density, the peripheral layers corresponding in density to those on the large fundal stone. On section the center of each of the four large stones consisted of a radiating, mottled, yellowish stone of cholesterol and a small amount of pigment with extensive cleft formation as shown in Figure 4. On the outside of each stone there were laminated deposits which varied in color with each stone. Those on the proximal ampullar stone were approximately the same color as its central portion but those on the other three stones were darker and the darkness increased in intensity proceeding from ampulla to fundus. The thirteen small fundal stones were dark in color and laminated. Apparently they were of recent origin and grew from the same materials as were laid down on the surface of the large fundal stone.

Samples for *chemical analysis* were taken from both peripheral and central portions of slices of the four large stones and from a ground-up small stone. The results of the analysis are shown in Table I. Since there was variation in appearance of different parts of the central and peripheral portions and since the samples were taken at random from these portions, a considerable degree of variation in chemical composition might be expected in corre-

sponding portions of the various stones. Calcium and phosphorus were present in the central portion of only the fourth or fundal stone and there only as a trace, while in the peripheral portion they were present in the second, third and fourth stones in increasing amounts away from the ampulla and in a small fundal stone they were present in greatest percentages. Bile pigment, the quantitative determination of which was the least reliable, was present in relatively small percentages in all of the stones and

TABLE I
Case 1

	Stone No. 1		Stone No. 2		Stone No. 3		Stone No. 4		Stone No. 5
	C*	P†	C	P	C	P	C	P	
Ether extraction (weight)	89.4%	64.6%	71.1%	72.9%	82.9%	83.7%	87.4%	63.8%	61.6%
Pure cholesterol (colorimeter)	65.2%	48.8%	34.3%	36.6%	56.2%	54.4%	79.4%	48.2%	46.8%
Calcium	0	0	0	trace	0	0.8%	trace	2.9%	4.4%
Phosphorus	0	0	0	trace	0	0.9%	trace	0.8%	2.8%
Pigment	0.1%	trace	0.2%	trace	0.1%	0.2%	0.2%	2.8%	0.5%
Dark residue	0.32%				1.89%				

* Center
† Periphery

there was no constant difference in distribution between central and peripheral portions although the highest percentage was present in the peripheral portion of the large fundal stone. Cholesterol was present in the peripheral portions in higher percentages than in the central portion except in stone No. 2 and there was no constant variation in cholesterol content of the stones progressing from ampulla to fundus.

The dark residue was determined on two samples; namely, one-half of stones Nos. 1 and 2 and one-half of stones Nos. 3 and 4 and one of the small stones. It was found to comprise by weight 0.32 per cent of the first sample and 1.89 per cent of the second sample which corresponds well with the gross appearance of the stones.

Case 2

Case 2 illustrates not only the presence of calcium salts and increased dark coloring matter in the peripheral layers of stones of the distal portion of the gallbladder, but also a large amount

of these substances in a stone located in a stagnant pocket off the middle portion.

A female, 50 years of age, had an attack of gallstone colic 1 month before examination. A small mass was palpable in the region of the gallbladder. A roentgenogram, Figure 5, revealed five radio-opaque, circular shadows in the region of the gallbladder, the second of which from the ampullar end was eccentrically located and more dense than the others. Attempted cholecystography resulted in nonvisualization of the gallbladder by the dye. Cholecystectomy was then performed. The gallbladder was thickened and elongated and was almost filled by a chain of stones, one of which protruded in a pocket near the middle portion. A roentgenogram of the unopened gallbladder (Fig. 6) revealed the shadows of seven stones. The two stones at the ampullar end cast practically no calcium shadows. The peripheral portion of the remaining stones cast calcium shadows, that of the pocketed stone being greatest in density.

The *gallbladder* was sectioned from the cystic duct to the fundus (Fig. 7). In addition to the stones it contained about 20 cc. of a cloudy mucoid, orange-colored fluid. The stones were faceted where they came in contact. All were pigmented and the chain increased in depth of color from ampulla to the fundus. However, the darkest stone of all was in the pocket at the middle portion of the chain where stagnation was apparently the greatest (Fig. 8). Section of the stones revealed greater density in the peripheral portion of those casting a calcium shadow in roentgenograms. The centers of all of the stones were similar in appearance, consisting of yellowish brown, radiating material about which there was a narrow, dark pigmented zone. The periphery of each was laminated with variation in color of the laminae. In general the periphery of each stone was increasingly dark in color from the ampulla to the fundus and that of the pocketed stone was darkest of all.

Chemical analyses were made of samples of the central and peripheral portions of slices of each stone (Table II). Again there was some irregularity in the variation but the darker stones toward the fundus and in the pocket contained greater amounts of calcium and phosphorus than the stones in the vicinity of the ampulla, and their peripheral portions contained greater amounts of these than the central portions. Pigment and dark residue were present in greater amounts in the stones that were darker in color.

TABLE II
Case 2

	Stone No. 1		Stone No. 2		Stone No. 3		Stone No. 4		Stone No. 5		Stone No. 6		Stone No. 7‡	
	C*	P†	C	P	C	P	C	P	C	P	C	P	C	P
Ether extraction (weight)	42.4%	67.5%	66.2%	70.4%	78.3%	88.2%	72.6%	72.8%	83.6%	74.1%	84.6%	77.3%	84.5%	71.9%
Pure cholesterol (colorimeter)	34.9%	44.4%	34.9%	51.3%	51.2%	55.8%	31.6%	43.6%	40.7%	39.6%	35.0%	48.5%	62.1%	46.5%
Calcium	trace	0.4%	trace	trace	trace	0.5%	trace	1.2%	trace	1.5%	trace	1.9%	0.4%	2.4%
Phosphorus	0	0	0	0	0	1.0%	0	1.1%	trace	trace	trace	1.0%	trace	1.3%
Pigments	0	trace	trace	trace	0.4%	0.3%	0.2%	0.5%	0.3%	1.0%	0.2%	1.1%	0.4%	1.0%
Dark residue	0.8%	0.3%	0	0.5%	0.4%	0.5%	2.5%	2.0%	1.1%	2.2%	1.6%	3.5%	1.0%	5.0%

* Center
† Periphery
‡ Pocketed stone

Case 3

A woman, 69 years old, a patient of Dr. G. M. Crabb, died of carcinoma of the colon.

At necropsy the *gallbladder* was found very large, thickened and filled with three large articulated stones. There were other small dark stones about the surface of the unusually large stone located in the fundus. The exterior of the stones was rough and dark brown, with the intensity of color slightly greater along the surface of the distal portion of the fundal stone (Fig. 9). A drawing of the sectioned stones is shown in Figure 10. The huge fundal stone contained a pure cholesterol stone as its central portion. This was doubtless the primary stone. Secondarily, the stones at the centers of the two others were laid down. Then came the laminar deposits on the three stones which were similar in appearance. The peripheral portion of the large distal part of the fundal stone was on the whole the darkest portion. A roentgenogram (Fig. 11) revealed the distribution of the more radio-opaque calcium shadows. There was a thin, irregular, radio-opaque ring about the periphery of the primary cholesterol stone and

about the two centers of the more proximately located stones. The periphery of the ampullar stone contained radio-opaque shadows along its articular surface opposite the ductal end. The middle stone also contained calcium shadows along its sides. The shadows of greatest density were in the periphery of the large fundal stone where the calcium salts had been deposited in blotches.

Case 4

In case 4 there was the usual difference in color in stones progressing from ampulla to fundus but calcium was present in extremely small amounts as judged by the roentgenogram.

A woman, 39 years old, had had occasional attacks of biliary colic over a period of 6 years. Physical examination was essentially negative. A roentgenogram revealed absence of radio-opaque shadows in the gallbladder region. Cholecystography showed visualization of the gallbladder which included a large oval, radiolucent mass. Cholecystectomy was performed.

The *gallbladder* showed slight inflammatory change and the cystic duct was patent. Three articulated stones were present which filled the gallbladder and increased in size and in intensity of color from ampulla to fundus (Fig. 12). The small ampullar stone was free of a surface coating but the second and third stones contained lamellar deposits which were brown in color and darkest on the fundal stone. The interior of each was similar to that of the ampullar stone. There were no radio-opaque shadows in the periphery of the fundal stone and only faint lines in its deeper portion indicative of very slight calcium deposition at an earlier stage in the stone formation.

In general these cases indicate that calcium deposition requires a higher grade of obstruction than is necessary for the laying down of the dark deposits. Also stagnation seems to favor the degradation of bile pigments into the dark compounds isolated by Aronson from gallstones. They are usually present in greatest amounts in those portions of stones that were laid down in fields of greatest stagnation.

SUMMARY

Cases are reported in which a small aggregation of similar gallstones, consisting principally of cholesterol, increased in size

and formed in a row filling a large part of the lumen of the gall-
bladder. Growth of the stones beyond that point caused par-
titioning of the lumen and stagnation of the bile in increasing
amounts in each compartment proceeding from ampulla to fundus.
With the further growth of the stones in these compartments
under conditions of differential stagnation, there resulted a dif-
ference in composition of the materials laid down simultaneously
on each stone. As stagnation increased from ampulla to fundus,
there was an increased tendency for the deposition on the stone of
each compartment of salts of calcium and phosphorus, of bile
pigment and of a dark material probably representing a degrada-
tion product of bile pigment. This differential deposit on the
stones showed a tendency to be increasingly dark in color and to
cast increasingly radio-opaque shadows in roentgenograms pro-
ceeding from ampulla to fundus of the gallbladder. The latter
finding is a point of value in diagnostic roentgenology. Stagna-
tion appears to be a factor in the formation of the dark con-
stituent of the gallstones.

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   in the cholesterol, bile pigment and calcium salts contents of gallstones
   formed in gallbladder and in bile ducts with the degree of associated
   obstruction. *Ann. Surg.*, 1939, **109**, 161–186.

2. Aronson, H. G. A component of gallstones insoluble in ordinary solvents
   and accounting in part for their dark coloration. *Arch. Path.*, 1940, **30**,
   670–674.

DESCRIPTION OF PLATES

PLATE 110

Fig. 1. Case 1. Radio-opaque shadows of stones in fundus of gallbladder.
Fig. 2. Case 1. Stones are shown in the opened gallbladder in (a) and after
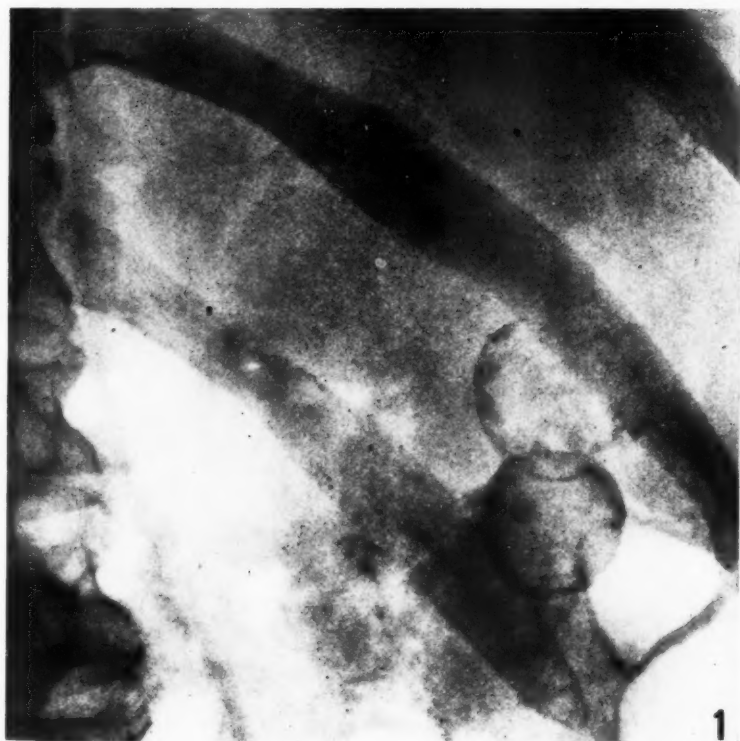removal in (b).

PLATE III

FIG. 3. Case 1. Roentgenograms of intact stones in (a) and of slices of four large stones and one intact small stone in (b). Calcium shadows in the two distal large stones and the small stones.

FIG. 4. Case 1. Reproduced from colored drawings of intact and sectioned stones. The peripheral layer of the stones is increasingly dark from ampulla to fundus. The centers of the large stones are of the same composition.
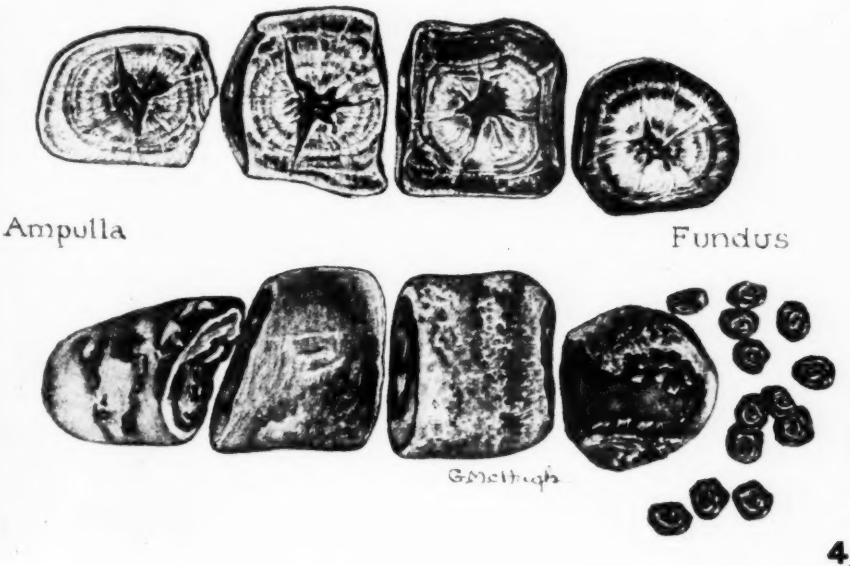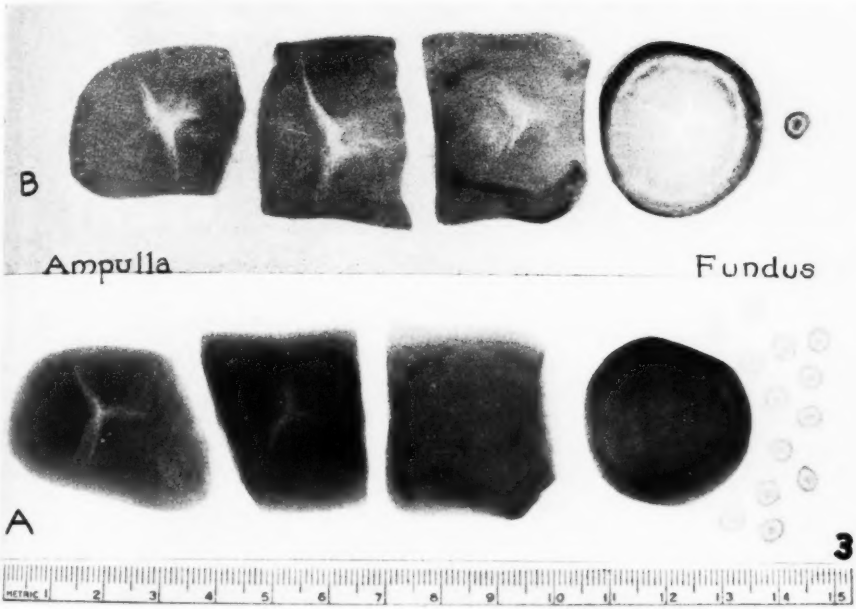
Ampulla

Fundus

Ampulla

Fundus

FIG. 5. Case 2. Ring-shaped radio-opaque shadows of stones in distal half of gallbladder and in pocket.

FIG. 6. Case 2. Roentgenogram of excised gallbladder before opening. There are ring-shaped radio-opaque shadows on the distal four stones, and heaviest on the pocketed stone. The two proximal stones do not show such ring-shaped shadows.
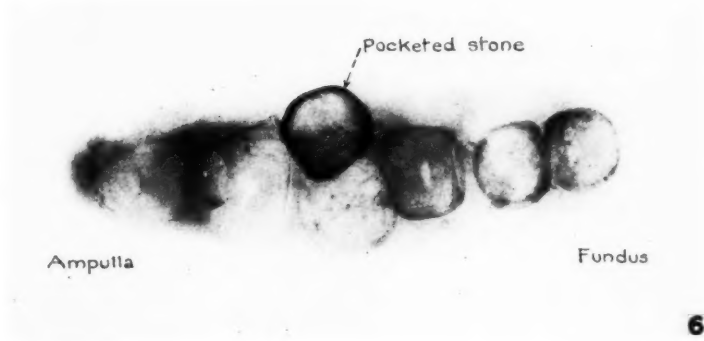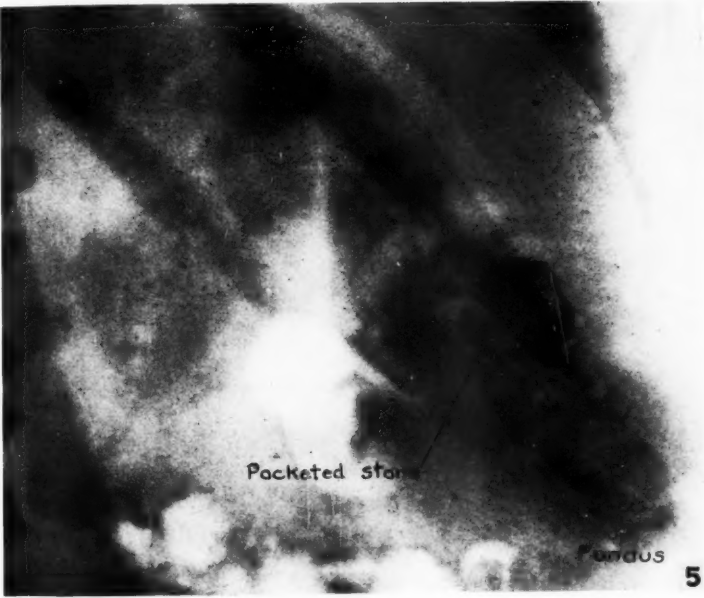
Pocketed stone

Fundus

5

Pocketed stone

Ampulla   Fundus

6

Phemister and Aronson   Composition of Gallstones

FIG. 7. Case 2. Incised gallbladder showing the stones in a pocket and in the fundal portion to be darker in color than those of the ampullar region.

FIG. 8. Case 2. Reproduced from colored drawings of exterior and cut section of stones, showing peripheral portions to be darkest in the stagnant fundus and in the pocket.
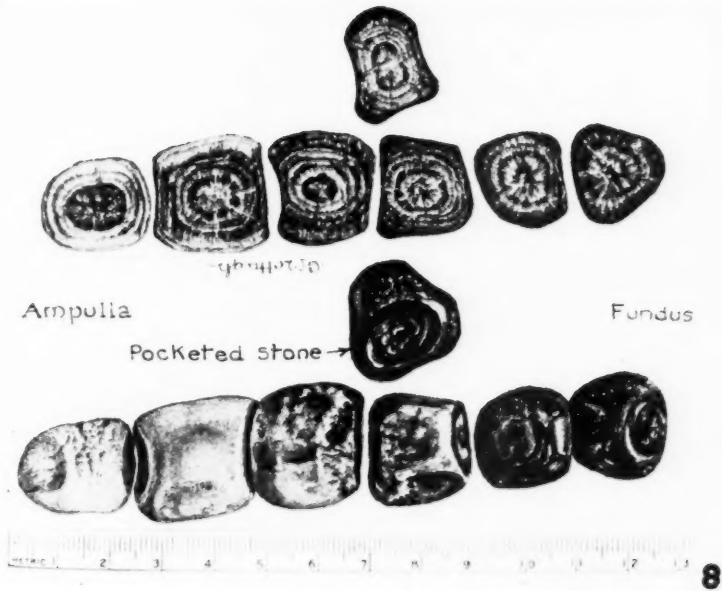
PLATE 113

A

PLATE 114

FIG. 9. Case 3. Articulated dark brown stones filling gallbladder.

FIG. 10. Case 3. Reproduced from a colored drawing of sectioned stones. The center of the large fundal stone contains a primary cholesterol stone. The centers of the two proximal stones are formed by cholesterol-pigment stones. Superimposed layers on all three stones are darkest in peripheral portion, and most of all in fundal stone.

FIG. 11. Case 3. Roentgenogram showing calcium shadows on distal end of ampullar stone, on periphery of middle stone and on all sides of large fundal stone. The primary cholesterol stone in the latter shows as a region of the least density.

PLATE 114

Ampulla

Fundus

9

Ampulla

Fundus

10

Ampulla

Fundus

Primary
cholesterol stone
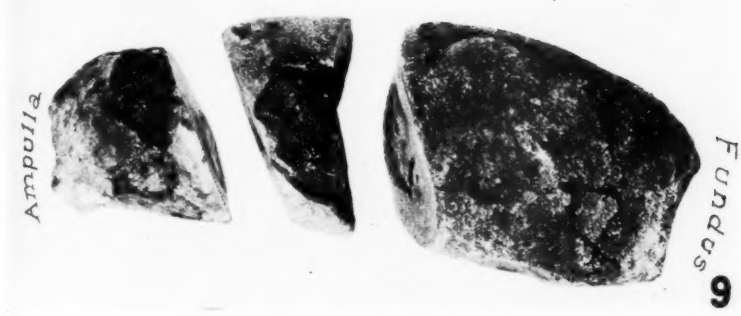
11

FIG. 12. Case 4. Reproduced from a colored drawing. There is an increasingly dark discoloration of the peripheral portion of the stones from ampulla to fundus. Ampullar stone is devoid of laminated peripheral deposit.

FIG. 13. Case 4. The roentgenogram shows absence of a calcium shadow in the periphery of the fundal stone.

Plate 115

Ampulla

12

Ampulla

13

Composition of Gallstones
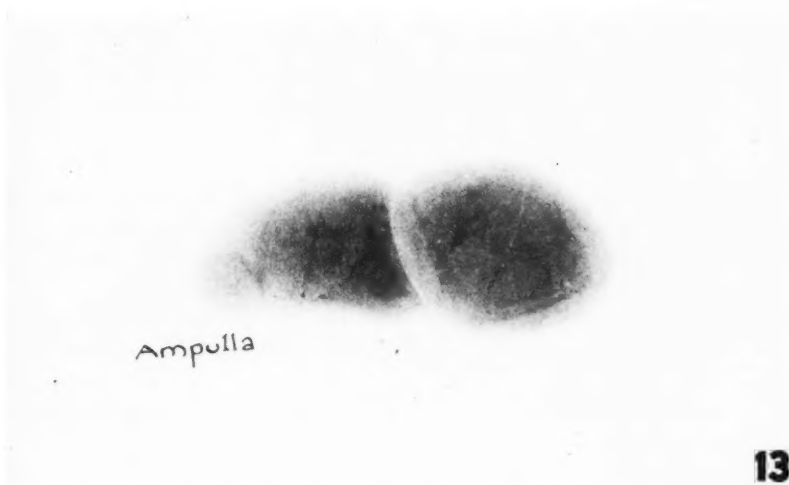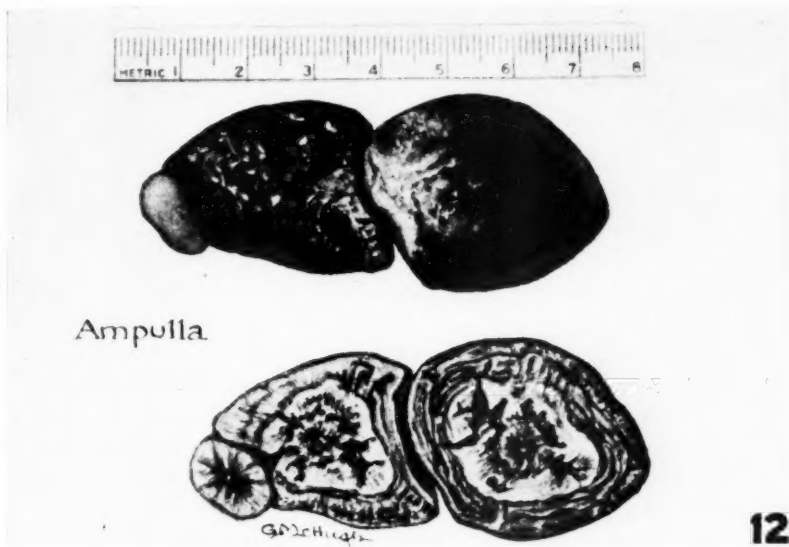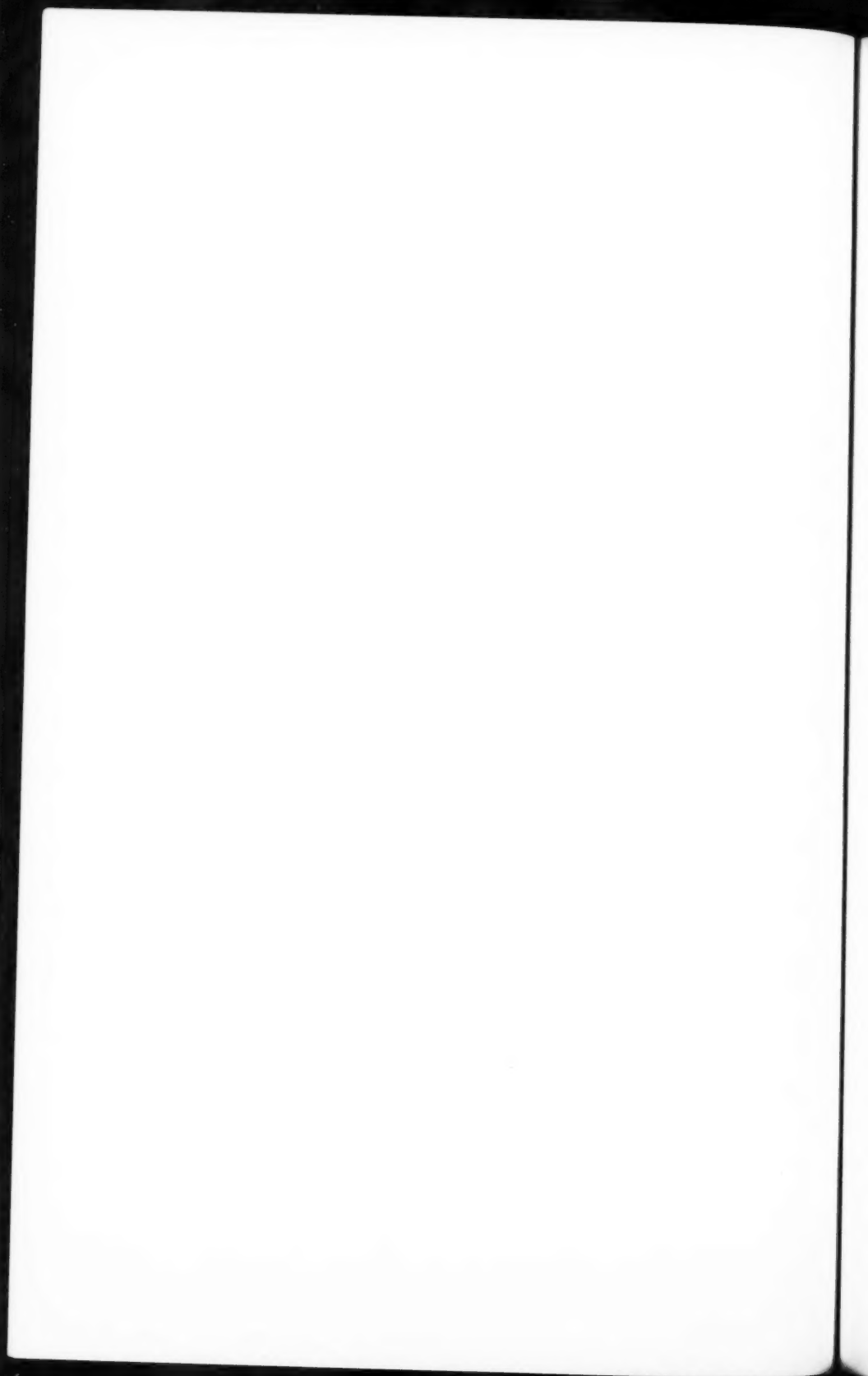
CERTAIN SPECIFIC AND IMMUNOPATHOLOGIC FEATURES OF TUBERCULOSIS *

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In earlier communications [1-3] studies were reported on the behavior of tubercle bacilli within the body. In 1936,[4] it was found that appropriate previous injections of avirulent human or bovine tubercle bacilli retard the development of subsequent infections with virulent human or bovine tubercle bacilli. Heat-killed avirulent and virulent tubercle bacilli exerted no such effect. Notwithstanding the facts that dissemination of virulent tubercle bacilli from the site of local intracutaneous inoculation is retarded in immune as compared with normal animals (demonstrated by Krause [5] and verified in this laboratory) and intravenous injection causes a widespread organic dissemination of the virulent bacilli, a definite retardation in the development of the organic disease was noted in immune animals infected intravenously as compared with control normal animals. As a result of quantitative evaluation of the bacilli and the disease,[6,7] it was found [8] that the bacillary body sensitized primarily to tuberculo-allergy and served to immunize against virulent infection. The natural filtrate from liquid cultures of tubercle bacilli on a simple synthetic nonprotein medium containing tuberculoprotein, however, sensitizes to anaphylaxis, provokes anaphylactic shock and allergic intoxication, but does not sensitize to allergy nor specifically immunize against virulent infection.

Evaluation of the effects of various procedures upon tuberculosis in the guinea pig has usually been made on the basis of one or both of two criteria: first the comparative extent of tuberculous involvement, giving, however, no information on life expectancy; and second and apparently of lesser value, the duration of life of the infected animals.

Recognizing the inherent difficulties, including the marked susceptibility of guinea pigs to infection by inoculation with highly virulent human tubercle bacilli, an investigation was planned to

study the effect of specific immunity to tuberculosis on a sufficiently extensive group of guinea pigs, and to compare them with a group of nonimmune guinea pigs kept under like conditions and infected with the same suspension of a culture of tubercle bacilli. While this work was being completed, Frappier and Forté [9] reported at the International Congress for Microbiology in 1939 that they had been able to prolong the life of immune guinea pigs and to prevent infection entirely in some of them.

The Specific Immune Feature

In order to note any effects of local spread as compared with general conditions in the animal economy, two routes of infection with the highly virulent human tubercle bacilli were chosen: subcutaneous and intravenous (Table I). The number of bacilli injected was also chosen to give the greatest amount of practical information both as to latitude and the time element. It is well known that there is a noticeable effect even with very large injections of virulent tubercle bacilli, since all the pioneering studies of Roemer, Krause, and Calmette were performed in that manner. It is also well known that inexactitudes of the physical factors involved in preparing properly homogeneous infecting materials are to be considered when very small amounts of highly virulent human or bovine tubercle bacilli are used. It is for this reason that complete prevention of infection by specific immunity could not be demonstrated entirely satisfactorily with the highly virulent bacilli used in these experiments and with the highly susceptible guinea pig as the test animal.

With subcutaneous injection (Table I) the immune guinea pigs lived on an average of 216 days while the nonimmune guinea pigs lived on an average of 101 days, both groups having received an infecting dose of 0.0001 mg. of bacilli. The results are even more striking with 0.000001 mg. of bacilli, in which case the immune guinea pigs showed an average duration of life of 319 days as compared with 127 days for the nonimmune guinea pigs. Although there is also a noticeable difference in the arbitrarily graded amount of macroscopic tuberculosis recorded, this alone can hardly present the entire picture of the existing condition. It must be appropriately qualified by statistics on the duration of life and also by the type of tuberculosis found at death. In the

TABLE I

Survival Time of Immune and Nonimmune Guinea Pigs Following Infection with Virulent Human Tubercle Bacilli

Immunized subcutaneously 1 month prior to infection	Tuberculin skin reaction 1:100 Seitz filtrate 2 days before infection	Infection virulent human tubercle bacilli (H 160)*	Survival time after infection in days and amount of tuberculosis (in brackets)	Average survival time after infection in days
1 mg. human avirulent tubercle bacilli	*grade* 3 in all†	0.0001 mg. subcutaneously	191 (4)‡ 270 (3) 253 (2) 97 (3) 273 (1)	216
Controls infected only	0 in all		92 (4) 103 (4) 123 (4) 87 (4) 99 (3)	101
1 mg. human avirulent tubercle bacilli	3 in all	0.000001 mg. subcutaneously	375 (4) 370 (4) 339 (3) 221 (3) 292 (2)	319
Controls infected only	0 in all		60 (4) 106 (4) 164 (3) 144 (4) 161 (4)	127
1 mg. human avirulent tubercle bacilli	3 in all	0.0001 mg. intravenously	145 (3) 147 (4) 155 (4) 122 (4) 177 (4)	149
Controls infected only	0 in all		73 (4) 70 (4) 52 (3) 68 (4) 52 (4)	63
1 mg. human avirulent tubercle bacilli	3 in all	0.0000001 mg. intravenously	243 (3) 217 (2) 187 (3) 267 (4) 288 (4)	240
Controls infected only	0 in all		90 (4) 86 (4) 197 (4) 97 (4) 103 (4)	114

* This strain of highly virulent human tubercle bacilli will infect nonimmune guinea pigs in amounts down to about one-billionth of a milligram.

† The skin tuberculin reaction is graded from 0 (no visible reaction beyond that obtained with an equivalent amount of salt solution) to 4 (a reaction 2 cm. in diameter with central necrosis).

‡ The anatomic tuberculous involvement is graded from 0 (no visible macroscopic tuberculosis in any of the organs) to 4 (a generalized marked involvement of all the important internal organs).

CORPER

immune guinea pigs a far less progressive type was noted, in so far as could be determined by macroscopic appearance and histologic evidence. As a whole, the more limited chronic forms of pathologic changes were found.

The findings recorded show also a relatively higher pathogenicity of the tubercle bacilli when infection is by the intravenous route of injection. Smaller amounts of bacilli thus given result in earlier death of the animal than is effected by the subcutaneous route. In spite of the general distribution of the bacilli by the intravenous route, these findings corroborate previously recorded results from this laboratory in that there is a striking effect of the specific immunity. This would seem to question the contention of earlier observers who believed immunity was mainly attributed to a local retardation of the spread of the bacilli as a result of the allergic condition. The average duration of life of the immune guinea pigs infected intravenously with 0.00001 mg. of bacilli was 149 days, as compared with 63 days for the nonimmune animals. Those infected intravenously with 0.0000001 mg. of virulent bacilli following immunization lived, on an average, for 240 days, as compared with 114 days for the nonimmune. The amount of anatomic tuberculosis, arbitrarily graded for tabulating purposes, showed also a definite retardation at the time of death in the immune guinea pigs as compared with the nonimmune. Both macroscopically and microscopically there was a more chronic, less progressive type of disease in the immune animals than in the controls, the process being similar to that found after subcutaneous injection. The pathologic characteristics are well shown in Figure 1 from a control guinea pig which died 103 days after virulent infection. In it a more diffuse type of disease was found in all the tuberculous organs, characterized by a more exudative nature, with a predominantly greater number of the younger types of monocytic cells and numerous granulocytic elements. In the immune animal, which lived 253 days after infection, the tuberculosis was more demarcated in the organs, and the cellular elements in the affected areas were of the more mature monocytic type, with fibroblastic tissue elements more conspicuous. The gross involvement also showed differences in the majority of cases in favor of the immune animals.

THE SPECIFIC ALLERGIC FEATURE

In a more exact analysis of tuberculosis, the individuality of the specific allergy as distinguished from specific immunity (to infection) must be appreciated, but how far and to what extent specific allergy plays a practical part in the disease has remained a disputed problem. To those who have assumed the normal presence or liberation of tuberculin in the tuberculous organism, the problem would appear to be more easily approached by studies with tuberculin; but to those who have sought more proof than the recovery of tuberculin from cultures or from postmortem materials, the way has not been so direct. Yet the experimental and practical use of tuberculin may throw some light on the problem if applied without carrying conclusions too far and with more exacting adherence to natural conditions. In a recently completed study [10] it was pointed out that, although the specific toxicity in tuberculosis cannot be described definitely as yet because of the lack of information regarding the part played by the products of tubercle bacilli liberated *in vitro* (natural filtrate containing tuberculoprotein—tuberculin) but apparently not liberated in appreciable amounts *in vivo,* desensitization with these products (tuberculoprotein) presents a fascinating problem in tuberculosis, the exact nature and significance of which will have to be disclosed by further experimental investigation. Evidences from the experimental study of tuberculo-anaphylaxis, tuberculo-allergy, and specific tuberculo-immunity would seem to permit question whether the active constituent of *in vitro* natural filtrate from the growth of tubercle bacilli is liberated *in vivo*. In spite of this, desensitization of bacillary tuberculo-allergically sensitized animals, prepared with either avirulent or virulent human tubercle bacilli, can be accomplished by appropriate treatment with natural tuberculo-filtrate (tuberculoprotein—tuberculin) when it is used in relatively small, primarily nontoxic, amounts. Animals thus prepared do not show a local specific skin reaction to natural filtrate (tuberculin) in customary amounts and are likewise protected against a lethal, general, allergic-shock intoxication. Tuberculo-desensitization with primarily nontoxic amounts of natural filtrate (tuberculoprotein) exerts no decided beneficial or detrimental effect upon the course of tuberculosis or upon specific tuberculo-immunity. The characteristics of the local tissue changes produced by tuberculin in the hypersensitive animals

have been frequently described [11-16] and range from acute edema
and cellulitis (granulocytic and monocytic) to profound necrosis
of tissue with all types of cellular repair ensuing subsequently,
the reaction depending on the hypersensitiveness and amount of
tuberculin. However, the organic toxic response in the hyper-
sensitive animal in the absence of local tuberculosis when pure
preparations from nonprotein mediums are used merits further
consideration, especially in elucidation of the final subject of
this study. A series of guinea pigs (Table II) was therefore
made allergically hypersensitive. Some of these were then de-
sensitized by appropriately spaced injections of a Seitz filtrate
prepared by growing tubercle bacilli on a nonprotein nutrient
medium (Wong-Weinzirl). The lungs, livers, spleens and kid-

TABLE II

*The Effect of Tuberculo-filtrate Treatment on General
Tuberculo-allergic Intoxication*

Interval between subcutane-ous injection of 1 mg. avirulent human tubercle bacilli and initiation of treatment with tuberculo-filtrate	Route of intoxicating injection* and inter-val after bacillary injection†	Results
1 month; then 6 weekly‡ intravenous injections of 1 cc. 1:10 filtrate	Intraperitoneally, 70 days	No lethal effect in any of the 5 guinea pigs in this series; a slight toxicity noted
No treatment		All 5 guinea pigs in this series died after 11 to 23 hours
6 weeks; then 5 intra-venous injections at in-tervals of 6 days, 1 cc. 1:10 filtrate	Subcutaneously, 70 days	No lethal effect in any of the 5 guinea pigs in this series; slight toxicity noted
No treatment		Lethal to all 5 guinea pigs in this series within 6 to 20 hours
1 month; then 5 subcutane-ous injections‖ at inter-vals of 6 days, 1 cc. 1:1 filtrate	Subcutaneously, 60 days	No lethal effect in 5 guinea pigs in this series; slight toxicity
No treatment		Lethal within 12 to 36 hours to 4 of 5 guinea pigs in this series; the other guinea pig recovered from a profound toxic reaction

* In all cases, 5 cc. natural filtrate (containing about 4 mg. tuberculoprotein) was used for
intoxicating. The intravenous route was not used in order to avoid anaphylactic shock.[8]
† The intoxicating injection of natural filtrate was given 4 to 6 days after the last filtrate
treatment (tuberculoprotein).
‡ The same effects were noted in the guinea pigs in which treatment was initiated coinci-
dently with the bacillary injection. These are omitted from the table.
‖ The animals treated intracutaneously showed about the same effects as those treated
subcutaneously.

neys of these animals were examined to note any differences between those dying from tuberculo-allergic intoxication and those protected by desensitization.

Gross examination disclosed distended emphysematous lungs in the animals which had succumbed to the allergic intoxication while the remaining organs appeared to be congested in variable degree, and there was a general congestion of all tissues other than the lungs. The livers particularly showed a distinct lobular pattern characteristic of passive congestion. The guinea pigs that recovered because of a previous protective treatment with filtrate (tuberculoprotein) showed no appreciable congestive reaction of the organs.

Histologic examination of sections of the lungs, livers, spleens and kidneys, stained by hematoxylin and eosin, disclosed the following in the animals that died following the allergic tuberculo-intoxication: The lungs revealed marked thinning of the alveolar walls with occasional apparent rupture, the nuclei stained well and there were no apparent changes in the bronchioli or bronchi. Occasionally the alveolar blood vessels appeared distended in certain areas. The liver sinuses in many of the animals appeared slightly distended and in certain areas the liver cell cytoplasm appeared granular and vacuolar. The nuclei of the liver cells stained normally, and the perilobular vessels as well as the centrilobular vessels were distended. In many cases the spleen revealed a mild distention of the pulp sinuses which were well demarcated. In the kidneys the main changes were found in the malpighian corpuscles in which the tuft vessels appeared to be distended and occasionally the intertubular vessels also were engorged. The nuclei appeared normal and no constant cytoplasmic changes were seen. In summarizing these findings, it should be noted that the predominant changes of the livers, spleens and kidneys of the guinea pigs killed by general tuberculo-allergy were those of congestion, while the lungs showed an acute or subacute emphysema.

For comparison, the organs of guinea pigs dying of acute tuberculo-anaphylactic shock were studied. As might be expected, it was found that the gross appearances in the lungs were much more striking in that distention and alveolar rupture were more common. The congestive mottling of the liver was less striking, with an apparent general engorgement of the entire organ. These studies seemed to indicate that the conspicuous histologic changes

noted did not concern the primary effect of the tuberculo-allergic intoxication but rather those secondary congestive changes consequent upon the general effects of the intoxication, resulting in acute or subacute emphysema of the lungs and circulatory stasis in the other organs. This is borne out by the sequence of events in the following experiments which point to the significance of this feature in the general picture of tuberculosis.

THE COMBINED FEATURES OF SPECIFIC IMMUNITY AND SPECIFIC TUBERCULO-ALLERGY IN TUBERCULOSIS ANALYZED IN A CONCRETE EXPERIMENT

Specific immunity can be demonstrated to exert a definite relative protection against tuberculosis in appropriate experiments, as was shown earlier in this paper and in previous publications. Specific tuberculo-allergy lacks significance in the specific immune effects, but plays a conspicuous rôle in the toxic (allergic) manifestations following the local or general application of the natural tuberculo-filtrate (tuberculoprotein—tuberculin). It may be questioned whether the latter is present at any time as such in the tuberculous economy. Therefore, it appeared desirable to plan an experiment which would more closely approach the conditions occurring naturally in the tuberculous organism and to disclose so far as possible the independence of these phenomena of specific immunity and specific allergy. A number of preliminary experiments showed that when guinea pigs were immunized with avirulent tubercle bacilli followed 1 month later by a large infecting intravenous injection of virulent tubercle bacilli, to imitate the natural mobilization of the bacilli in the tuberculous, the results were rather disastrous. In many cases, the immune guinea pigs suffered an acute or subacute allergic death, while the controls would usually survive for several weeks and then would all succumb within a short interval, to be outlived considerably by all the immune guinea pigs that had not succumbed to the early allergic consequences.

As a result of these preliminary tests and those cited earlier in this paper, the following experiment was performed and has been duplicated since. Twenty-four male guinea pigs of about equal size were divided into three groups of 8 each. Those in group 1 were used as *controls*, and the animals were infected by an intravenous injection of 1 mg. of highly virulent human tubercle bacilli

(H 160) in fine suspension 32 days after initiation of the experiment. Group 2, *specific immune*, was given a subcutaneous injection of 1 mg. of avirulent human tubercle bacilli 32 days prior to infection by the intravenous injection of 1 mg. of virulent human tubercle bacilli (H 160). Group 3, *immune and treated*, was given the subcutaneous injection of 1 mg. of avirulent human tubercle bacilli and treated 7 days later by injecting 1 cc. of 1:10 dilution of natural filtrate, which was repeated every fifth day, a total of 5 injections, until the 27th day, and on the 32nd day after immunization they were infected by being given intravenously 1 mg. of virulent human tubercle bacilli (H 160) in fine suspension. The weight curves of these three groups of animals, the mortality figures and pathologic findings all proved highly enlightening. The mortality figures in days will be presented briefly and the significant findings given.

The duration of life after virulent infection for the *controls* was 11, 15, 16, 16, 17, 18, 18 and 18 days, with an average duration of 16 days; the *specific immune* guinea pigs, on the other hand, lived 19 hours, 36 hours, 8, 9, 17, 19, 22 and 28 days after virulent intravenous infection, with an average life duration of 13 days; and the *specific immune and treated* guinea pigs lived 17, 19, 22, 23, 23, 24, 26 and 27 days, with an average duration of life of 23 days. It is to be noted that the natural filtrate treatment for the specific tuberculo-allergic hypersensitiveness of the specifically immunized guinea pigs prolonged life following the intravenous infection with the virulent human tubercle bacilli from an average of 13 to 23 days by preventing the lethal allergic intoxication usually resulting from such virulent infection (mobilization of bacilli?). The specific immunity prolonged the life of the immune guinea pigs, as compared with the nonimmune controls, from an average of 16 to 23 days.

Pathologic Findings

The gross and microscopic findings in the lungs, livers, spleens and kidneys of these animals will be presented briefly. The organs were sectioned and stained with hematoxylin and eosin for histologic study as well as with carbol fuchsin for noting tubercle bacilli. Aside from the findings recorded (Table III), detailed pathological description yielded little unified significant information.

TABLE III
Pathological Findings

Animal and time of death in days	Lungs	Liver	Spleen
Control 11	Slightly distended; congested; tubercles (?). *Pneumonitis; congested; transudate; tubercle bacilli, 2.*	Pale; lobules prominent. *Fatty infiltration, 4; tubercles, 1; tubercle bacilli, 1.*	Measured 4×1.5 cm.; congested; tubercles, 0. *Tubercles, 2; congested, 2; tubercle bacilli, 2.*
Control 15	Distended; tubercles, 2. *Pneumonitis; transudate; tubercles, 2; tubercle bacilli, 4.*	Pale; lobules prominent. *Fatty infiltration, 3; tubercles, 2; tubercle bacilli, 1.*	Measured 3.5×2 cm.; congested; tubercles, 0. *Tubercles, 2; congested, 2; tubercle bacilli, 3.*
Control 16	Distended; tubercles, 3. *Tubercles, 3, necrotic; transudate; tubercle bacilli, 4.*	Pale; lobules prominent. *Fatty infiltration, 1; tubercles, 2; tubercle bacilli, 4.*	Measured 5×2.5 cm; tubercles(?). *Tubercles diffuse; congested; tubercle bacilli, 4.*
Control 16	Distended; tubercles, 4. *Pneumonitis; slightly congested; transudate; tubercle bacilli, 3.*	Pale; lobules distinct. *Fatty infiltration, 4; tubercles, 2; tubercle bacilli, 1.*	Measured 4×2 cm.; tubercles, 2. *Tubercles general; congested; tubercle bacilli, 2.*
Control 17	Distended; tubercles, 3. *Pneumonitis; transudate; tubercles, 2; tubercle bacilli, 3.*	Pale; lobules prominent. *Fatty infiltration, 2; tubercles, 2, necrotic; tubercle bacilli, 2.*	Measured 5×2 cm.; tubercles(?). *Tubercles diffuse; congested; tubercle bacilli, 1.*
Control 18	Distended; tubercles confluent. *Pneumonitis; tubercles, 2, necrotic; tubercle bacilli, 4.*	Pale; lobules prominent. *Fatty infiltration, 2; tubercles, 1, necrotic; tubercle bacilli, 3.*	Measured 3.5×2 cm.; tubercles, 0. *Tubercles diffuse; congested; tubercle bacilli, 3.*
Control 18	Distended; tubercles, 4. *Pneumonitis; transudate; tubercles necrotic; tubercle bacilli, 2.*	Brown; lobules distinct. *Fatty infiltration, 1; tubercles, 2; tubercle bacilli, 2.*	Measured 4×2 cm.; tubercles, 2. *Tubercles diffuse, necrotic; tubercle bacilli, 3.*
Control 18	Distended; tubercles, 4. *Pneumonitis; transudate; tubercles necrotic; tubercle bacilli, 4.*	Brown; lobules prominent. *Fatty infiltration, 1; tubercles, 1, necrotic; tubercle bacilli, 3.*	Measured 4×2 cm.; tubercles, 0. *Tubercles diffuse, necrotic; tubercle bacilli, 3.*

Group	No.			
Immune	1	Distended; dark; congested; tubercles, o. *Congested; hemorrhage; tubercles, o; tubercle bacilli, 1.*	Dark; discrete yellow mottling. *Congested; tubercles, o; tubercle bacilli, o.*	Measured 2×1.5 cm.; congested; tubercles, o. *Congested; tubercles, o; tubercle bacilli, o.*
Immune	2	Distended; dark; congested; tubercles, o. *Pneumonitis; congested; slight hemorrhage; tubercle bacilli, 1.*	Brown; irregular mottling. *Granulation; tubercles, o; tubercle bacilli, 1.*	Measured 2×1.5 cm.; congested; tubercles, o. *Congested; tubercles, o; tubercle bacilli, 1.*
Immune	8	Distended; tubercles, o. *Pneumonitis; congested; transudate; tubercles, o; tubercle bacilli, 2.*	Yellow; mottled. *Fatty infiltration, 1; tubercles, 1; tubercle bacilli, o.*	Measured 3×2 cm.; congested; brown; tubercles, o. *Tubercles, 2; tubercle bacilli, 3.*
Immune	9	Distended; tubercles(?). *Pneumonitis diffuse; hemorrhage; transudate; tubercle bacilli, 2.*	Yellow; mottled. *Fatty infiltration, 3; tubercles, 1; tubercle bacilli, 1.*	Measured 3×1.5 cm.; tubercles confluent. *Congested; tubercles diffuse; tubercle bacilli, 3.*
Immune	17	Distended; tubercles confluent. *Congested; tubercles, 3; necrotic; transudate; tubercle bacilli, 4.*	Yellow; mottled. *Fatty infiltration, 3; tubercles necrotic, hyaline; tubercle bacilli, 3.*	Measured 3.8×2 cm.; areas of massive necrosis. *Tubercles, 3, necrotic; congested; tubercle bacilli, 4.*
Immune	19	Distended; pale; tubercles(?). *Pneumonitis, 2; transudate; tubercles, 3; necrotic; tubercle bacilli, 3.*	Yellow; mottled. *Fatty infiltration, 4; tubercles, 2; tubercle bacilli, 2.*	Measured 6.5×3 cm.; diffuse; necrotic; congested. *Congested, 4; tubercles, 3; necrotic; tubercle bacilli, 3.*
Immune	22	Distended; tubercles discrete. *Pneumonitis, 4; congested, 2; necrotic; tubercle bacilli, 3.*	Yellow; mottled. *Fatty infiltration, 1; congested, 3; tubercles, 2; necrotic; tubercle bacilli, 2.*	Measured 4×2.5 cm.; massive necrosis. *Necrotic, 4; tubercle bacilli, 2.*
Immune	28	Distended; tubercles discrete multiple. *Pneumonitis, 2; tubercles, 3; necrotic; tubercle bacilli, 4.*	Brown; mottled yellow. *Fatty infiltration, 1; tubercles, 1, necrotic; congested, 1; tubercle bacilli, 4.*	Measured 5×2.5 cm.; dark; multiple necroses. *Congested, 2; tubercles, 2; necrotic; tubercle bacilli, 2.*
Treated immune	17	Distended; tubercles, o. *Pneumonitis, 2; tubercles, 2; transudate; tubercle bacilli, 2.*	Light brown; discrete mottling. *Fatty infiltration, o; tubercles necrotic and hyaline, 1; congested; tubercle bacilli, 2.*	Measured 4×2 cm.; dark mottled yellow. *Congested, 2; tubercles necrotic and hyaline, 3; tubercle bacilli, 2.*

TABLE III (Continued)

Animal and time of death in days	Lungs	Liver	Spleen
Treated immune 19	Slightly distended; tubercles(?). *Pneumonitis, 4; transudate, 1; tubercle bacilli, 4.*	Brown; mottled. *Fatty infiltration, 2; tubercles necrotic and hyaline, 2; congested, 2; tubercle bacilli, 2.*	Measured 5.5×2.5 cm.; brown mottled. *Congested, 4; tubercles necrotic and hyaline, 2; tubercle bacilli, 3.*
Treated immune 22	Distended; tubercles, 3; *Pneumonitis diffuse, 2; tubercles, 2; congested, 2; tubercle bacilli, 3.*	Yellow; mottled. *Fatty infiltration, 1; tubercles, 2, necrotic; congested, 2; tubercle bacilli, 2.*	Measured 4×2 cm.; brown; diffuse. *Congested, 3; tubercles necrotic and hyaline, 3; tubercle bacilli, 3.*
Treated immune 23	Distended; tubercles diffuse. *Pneumonitis diffuse, 3; tubercles, 1; congested, 1; tubercle bacilli, 4.*	Yellow; mottled. *Fatty infiltration, 2; tubercles necrotic; congested, 1; tubercle bacilli, 1.*	Measured 4×2 cm.; brown; mottled. *Tubercles necrotic and hyaline; congested, 2; tubercle bacilli, 3.*
Treated immune 23	Distended; tubercles diffuse. *Pneumonitis diffuse, 2; congested, 2; transudate, 1; tubercle bacilli, 3.*	Brown; yellow mottled. *Fatty infiltration, 3; tubercles, 2 necrotic; tubercle bacilli, 1.*	Measured 4×2 cm.; brown; diffuse mottling. *Tubercles necrotic and hyaline, 3; congested, 3; tubercle bacilli, 3.*
Treated immune 24	Distended; tubercles diffuse. *Pneumonitis diffuse, 2; tubercles, 2; tubercle bacilli, 4.*	Light brown; yellow mottling. *Fatty infiltration, 1; tubercles, 3; necrotic and hyaline; tubercle bacilli, 3.*	Measured 5×2.5 cm.; diffuse; necrotic. *Tubercles, 3, necrotic and hyaline; congested, 3; tubercle bacilli, 3.*
Treated immune 26	Distended; slight mottling. *Pneumonitis diffuse,3; tubercles necrotic, 2; congestion and hemorrhage, 2; tubercle bacilli, 3.*	Light brown; yellow mottling. *Fatty infiltration, 1; tubercles necrotic, 2; tubercle bacilli, 2.*	Measured 4×2 cm.; dark brown; yellow; necrotic. *Tubercles necrotic and hyaline, 2; congested, 2; tubercle bacilli, 3.*
Treated immune 27	Distended slightly and mottled. *Pneumonitis diffuse, 3; congested, 1; tubercles, 3; hemorrhage and transudate, 2; tubercle bacilli, 4.*	Brown; slightly mottled. *Fatty infiltration, 0; tubercles necrotic, 2; tubercle bacilli, 1.*	Measured 4×2.5 cm.; congested and mottled. *Tubercles necrotic and hyaline, 3; congested, 2; tubercle bacilli, 2.*

The numerals from o to 4 signify the approximate arbitrary grading of the finding. Macroscopic findings are listed in ordinary type; microscopic findings in italics.

In analyzing the pathologic information disclosed in the above experiment, several phases are found which merit consideration. The animals that died early as a result of the intravenous injection of the large amount of virulent human tubercle bacilli showed, as would be expected, very little discrete tuberculosis in the lungs, liver and spleen. But they did show predominant evidences of an acute toxicity centered about the general allergic reaction. Yet this was not sufficiently pronounced to present the characteristics of a local reaction resulting from the injection of a sufficient amount of tuberculin to provoke evident tissue changes. The changes noted proved to have more of a secondary character (pulmonary emphysema and congestion of the other organs) in the animals dying early, while later the reaction to the bacilli in the organs obscured this picture so that its recognition was impossible.

It can be assumed, however, from other evidence presented, that the animals that died within the first 10 days following intravenous injection of the virulent bacilli died either from the consequences of the violent specific allergic response or the combination of this with the rapidly developing tuberculosis. This is also borne out by the fact that the controls all died after 11 days and within the short span of 15 to 18 days in most cases, although the part played by specific allergy is difficult to determine. However, when specific allergy in its more active form has subsided as in the immune animals presenting a longer interval, or when it has been suppressed as in the treated immune animals, the predominant reaction seen pathologically is that of the ultimately lethal tuberculosis. The pathological findings in the lungs, livers and spleens at death in the immune or the treated immune guinea pigs do not differ materially from those in the normal infected animals except in so far as can be accounted for by the time that death occurred. Even the bacillary findings cannot be interpreted in this light as significant if we view them with respect to the technical difficulties encountered in such examinations. However, the period of death in this experiment is significant in pointing out the part played by the specific immunity in prolonging life, particularly after the specific allergy has been suppressed in the treated immune animals. As has been shown previously, had the animals all been examined at a certain definite

interval, the pathological findings would have shown striking differences. What stands out strikingly here, however, is the fact that in spite of the overwhelmingly large intravenous injection used as the infecting dose in this experiment, specific immunity displayed a definite retarding effect on the lethal factors involved in tuberculosis, resulting in prolongation of life. These latter infecting injections were required to bring out the allergic response with bacillary suspension, and the intravenous injection of o.oi mg. of virulent bacilli resulted in no allergic deaths. It also appears that the general lethal allergic manifestations do not become significant, at least experimentally, until relatively large amounts of bacilli are involved in the animal economy. Whether general allergy does or does not play a significant part in spontaneous tuberculosis in man is involved with the problem whether the proper antigenic intoxicating materials in man are liberated (or mobilized) *in vivo*, such as the proper mobilization of bacilli or the liberation of tuberculoproteins. The present evidence would not appear to support the contention that tuberculin (or tuberculoproteins) as prepared *in vitro* are liberated in active form *in vivo*, although much has been written on auto-inoculation in the past.

Summary and Conclusions

The life expectancy of guinea pigs infected subcutaneously or intravenously with highly virulent human tubercle bacilli is prolonged considerably by specific immunization. When large intravenous infecting doses of virulent tubercle bacilli are used, general specific allergic intoxication becomes a significant factor causing an early lethal outcome for the specific immune animals and decidedly lowering the average life span after infection for these animals. By appropriate treatment with suitable filtrates (or pure tuberculins), derived from growing tubercle bacilli on nonprotein mediums, these undesirable allergic lethal intoxications can be prevented. Such treated animals then display only the specific immune protection with resultant retardation of the tuberculosis and prolongation of life. A classical experiment demonstrating the significance of suppressing the specific tuberculo-allergy by appropriate treatment, with resulting prolongation of life of specific immune guinea pigs infected intravenously with virulent tubercle bacilli, is presented. The various phases of the

pathology of these conditions in this experiment are also presented. The visual pathological changes at the time of death of these animals disclose only the secondary changes resulting from the specific tuberculo-allergic intoxication on the one hand and the specific tuberculous pathology on the other, unless the time element and other findings are correlated to bring out the significance of the specific immune factors involved. Thus the predominant pathological features noted are the secondary changes produced by the specific tuberculo-allergy, following the second introduction of relatively large amounts of bacillary bodies, and the pathological changes characteristic of tuberculosis as usually recognized.

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DESCRIPTION OF PLATE

PLATE 116

The organic tuberculous involvement at the time of natural death in non-immune and specific immune guinea pigs infected subcutaneously with highly virulent human tubercle bacilli (strain 160).

FIG. 1. Control guinea pig, died 103 days after infection with 0.0001 mg. virulent human tubercle bacilli.

FIG. 2. Specific immune guinea pig (prepared by a single subcutaneous injection of avirulent human tubercle bacilli) and infected as was the animal used for Figure 1. This animal died 253 days after infection. Less extensive and more discretely demarcated tuberculosis is shown in the immune animal (Fig. 2) than in the nonimmune control (Fig. 1).

PLATE 116
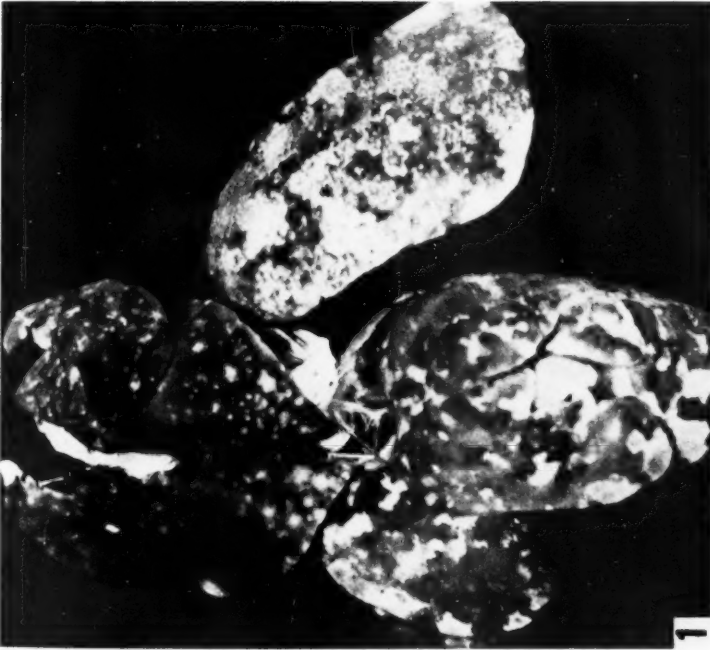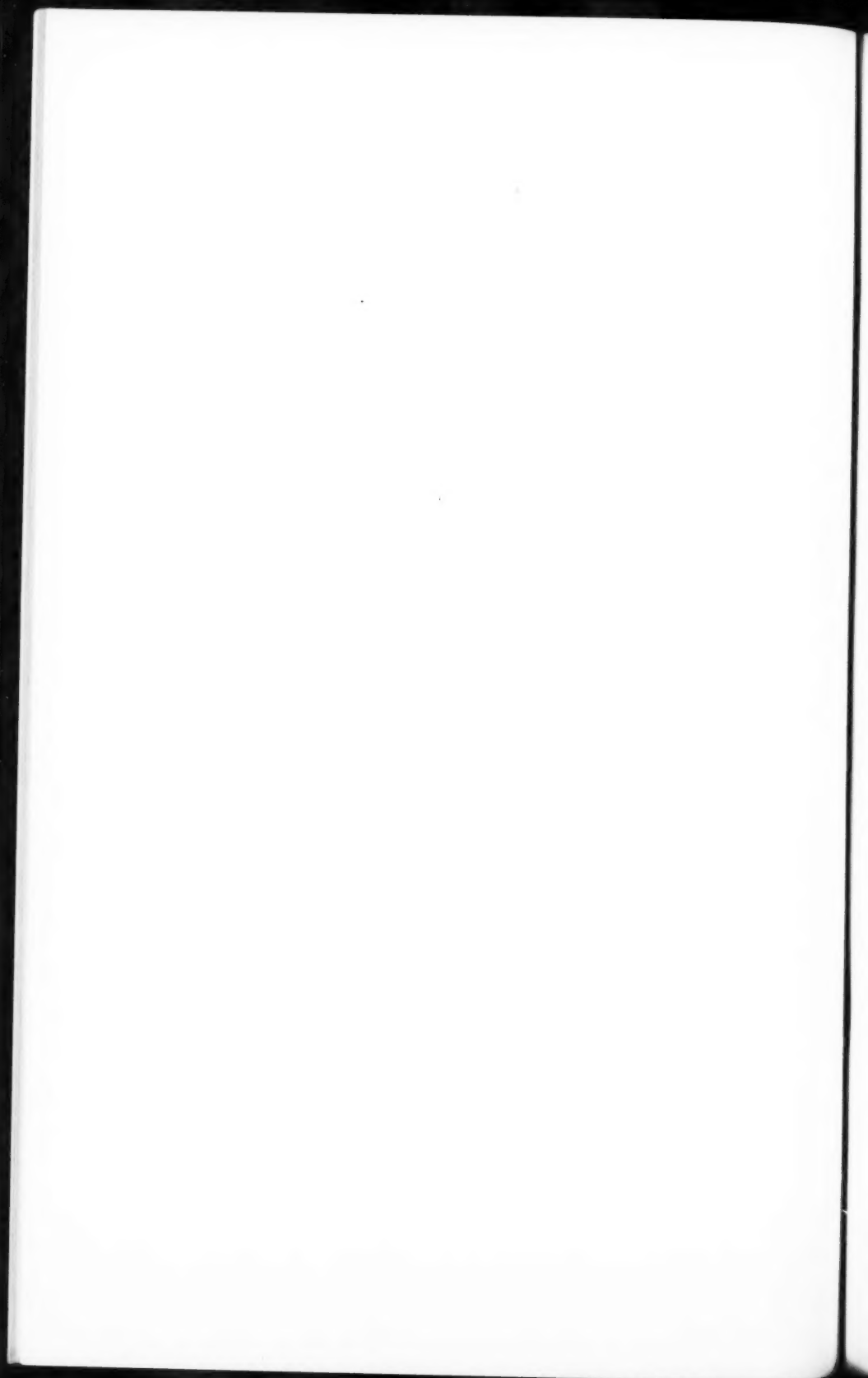
THE SPREAD OF TUBERCLE BACILLI BY SPUTUM, BLOOD AND LYMPH IN PULMONARY TUBERCULOSIS *

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Tuberculosis is well generalized in advanced pulmonary tuberculosis. The fact is recognized by most pathologists although minimized in the Ranke concept of tuberculosis, in which the disease is divided into stages, the third of which, typified by phthisis, is defined as isolated organ tuberculosis. The wide generalization is usually unrecognized clinically.

Tubercle bacilli are carried from the lungs in advanced pulmonary tuberculosis by sputum, blood and lymph. A common site of tuberculosis secondary to pulmonary disease, but one that is seldom recognized in life, is the tonsil. One or both members of the pair are tuberculous in the great majority of cases of pulmonary tuberculosis. The lymph nodes tributary to the tonsils are also usually the seat of tubercles in pulmonary tuberculosis. Microscopic study shows minute tubercles in the viscera, often on a scale not greatly different from that in clinical miliary tuberculosis, as well as tuberculosis of the intestines and varying degrees of involvement of the internal and external lymph nodes. Any of the organs named could be infected by either blood or lymph, and two of them, the tonsils and intestines, could be infected by sputum. It has recently been shown that a large proportion of the cases of tuberculosis of the tonsil discovered on the routine examination of this organ after tonsillectomy really represent spread from unrecognized pulmonary tuberculosis.[1]

Starting from the latter observation, we have made a study of the several routes of spread by correlating the incidence in selected groups of organs. The material for investigation came from 126 cases of fatal pulmonary tuberculosis, almost exclusively of the common advanced chronic form, occasionally complicated by clinical miliary disease. The 126 cases were selected from a larger group after exclusion of those in which tonsils could not be found on gross examination. The groups of organs set up

for comparison were selected to test the probability of spread by the several routes mentioned. Since the extent of generalization is popularly believed to be much greater in Negroes than whites, figures were calculated for the races separately in certain of the correlations. The first correlations were to throw light on the amount of spread by passage of infected sputum over the organs concerned. Routinely only one good section of each organ was examined. Additional sections would almost certainly have increased the number of tubercles found. Hence the figures given are minimal.

CORRELATION OF TONSILLAR AND INTESTINAL TUBERCULOSIS

In 126 cases of pulmonary tuberculosis in which the tonsils were examined they were found to be tuberculous in 93 (74 per cent). In 107 cases in which both tonsils and intestines were examined the latter were tuberculous in 84 (79 per cent). Among the 84 cases in which the intestines were tuberculous the tonsils contained tubercles in 68 (81 per cent). In 16 (19 per cent) of the intestine-positive cases no tubercles were found in the tonsils. In the 23 cases in which tuberculosis was not found in the intestines the tonsils were positive for tuberculosis in 57 per cent and negative in 43 per cent of cases. Thus there was a significantly higher proportion of tonsil-positive cases among the intestine-positive than in the intestine-negative cases; yet numerous cases of tuberculosis of the tonsils were found in the absence of tuberculosis of the intestines.

In the 107 cases in which intestines and tonsils were examined the tonsils were tuberculous in 81 (76 per cent) and no tuberculosis was detected in 26 (24 per cent). Among the 81 cases in which the tonsils were positive the intestines were tuberculous in 84 per cent and negative in 16 per cent. In the 26 cases in which tuberculosis was not found in the tonsils the intestines were positive for tuberculosis in 62 per cent and negative in 38 per cent. Thus the percentage of cases with intestinal tuberculosis was significantly higher in the tonsil-positive than in the tonsil-negative group; yet there were numerous cases of tuberculosis of the intestines in the absence of tuberculosis of the tonsils. It must be recalled that the figures are based on gross examination and a single microscopic section from each organ.

Correlation of Tuberculosis of Tonsils and of Upper Cervical Lymph Nodes

In 122 cases in which both the tonsils and the upper cervical lymph nodes were examined, tuberculosis was found in the former in 91 cases (75 per cent) and in the latter in 85 (70 per cent). Among the 91 tonsil-positive cases tuberculosis was discovered in the corresponding upper cervical lymph nodes in 76 (83 per cent) and not found in 15 (17 per cent). In the 31 cases in which the tonsils were negative the upper cervical lymph nodes were positive for tuberculosis in 9 (29 per cent) and negative in 22 (71 per cent). Thus tuberculosis was found in the cervical lymph nodes in the majority of cases in which it was present in the tonsils, and in the great majority of cases was not found in the cervical lymph nodes when it was absent in the tonsils.

Among the 85 cases in which the cervical lymph nodes were tuberculous, tuberculosis was discovered in the corresponding tonsils in 76 (89 per cent) and not found in 9 (11 per cent). Among the 37 cases in which tuberculosis was not found in the upper cervical lymph nodes, tubercles were discovered in the tonsils in 15 (41 per cent) and not found in 22 (59 per cent). Thus tuberculosis was present in the tonsils in only a minority of cases in which it was absent in the upper cervical lymph nodes.

Correlation of Tuberculosis of Intestines and of Mesenteric Lymph Nodes

In 92 cases in which both intestine and mesenteric lymph nodes were examined tuberculosis was found in the former in 74 (80 per cent) and in the latter, also, in 74 (80 per cent). Among the 74 intestine-positive cases tuberculosis was found in the mesenteric lymph nodes in 66 (89 per cent) and not found in 8 (11 per cent). Among the latter were several cases with such extensive amyloidosis that tuberculosis could not have been detected in the lymph nodes. In the 18 cases in which tuberculosis was not found in the intestine the mesenteric lymph nodes contained tubercles in 8 (44 per cent) and no tuberculosis was found in 10 (56 per cent). Thus tuberculosis was twice as frequent in the mesenteric lymph nodes in cases of intestinal tuberculosis as in cases free from intestinal tuberculosis.

The figures were identical for the opposite correlation of tuberculosis of the intestine and of the mesenteric lymph nodes. Among the 74 positive cases for the mesenteric lymph nodes, tuberculosis was found in the intestine in 66 (89 per cent) and not found in 8 (11 per cent). In the 18 cases in which tuberculosis was not found in the mesenteric lymph nodes, intestinal tuberculosis was present in 44 per cent and absent in 56 per cent. Thus tuberculosis was twice as frequent in the intestine when the mesenteric lymph nodes were tuberculous as when tuberculosis was not found in the nodes.

If it is assumed that intestinal tuberculosis as a complication of advanced pulmonary tuberculosis results as a rule from infection by swallowed sputum, and that tuberculosis of the mesenteric lymph nodes in such cases is secondary to the intestinal disease, resulting from spread by the lymphatics, the figures cited in previous paragraphs indicate equal reason for believing that tonsillar tuberculosis in the presence of pulmonary tuberculosis results from sputum infection and that accompanying tuberculosis of the upper cervical lymph nodes is the result of spread by way of the regional lymphatics from the tonsil to the nodes. Certain European investigators consider tonsillar tuberculosis as usually hematogenous.[2]

However, in both instances involvement of the tributary organ was noted in the absence of recognized involvement of the source organ. This might have been due to failure to detect the disease in the latter organ, or it might have been from other cause, namely, infection of the respective lymph nodes by another route. The other possible routes are lymphatic from a more distant focus, and hematogenous. The next correlation to be considered throws light on the problem.

CORRELATION OF TUBERCULOSIS OF TONSILS, UPPER CERVICAL LYMPH NODES AND PARATRACHEAL, AXILLARY AND INGUINAL LYMPH NODES

A comparison was made of the incidence of tuberculosis in five lymphoid structures; namely, the tonsils, and the upper cervical, paratracheal, axillary and inguinal lymph nodes. It was assumed that any of these organs might be infected by way of the blood stream and that the various nodes might also be

infected by extension through the lymphatics from neighboring infected organs. Specifically the upper cervical nodes might be infected from the tonsils and the paratracheal and axillary nodes from the lungs. The paratracheal nodes selected were those at the level of the clavicle, still in the line of drainage from the lungs to the thoracic duct. Infection other than hematogenous would be from lymph that had already passed through several lymph node filters, which in turn had been infected. Axillary lymph node infection might be hematogenous or the result of spread of tuberculosis through the lymphatics of pleural adhesions. Bilateral pleural adhesions were practically universal in the series of cases studied. But, if tuberculosis could spread by this route to the axillary nodes, it could spread to the neck by the same route and infect the lower cervical nodes. With infection of the lower nodes, disturbance in flow with retrograde diversion of lymph might occur, leading to infection of the upper cervical nodes. Thompson,[2] quoting the pertinent literature, has described this route of infection in more detail. Thus it is conceivable that tuberculosis of the latter group might result by lymphatic spread from the lungs. With such considerations in mind it seemed desirable to make a unilateral comparison of the incidence of tuberculosis in the axillary and upper cervical nodes.

Of all the lymph node groups selected the one most nearly representing hematogenous infection alone was the inguinal group. The inguinal nodes could be, and several times in the series were, infected as part of a generalized lymphatic dissemination, but for the most part were uninfected or were the seat of isolated tuberculosis of blood-borne origin. It is recognized that occasionally hematogenous infection might be indirect, by lymphatic spread from a part below the groin previously infected hematogenously, and that the lymphatics of a very small portion of the lower bowel drain to the superficial inguinal lymph nodes.

All five groups of organs, *i.e.*, tonsils and upper cervical, paratracheal, axillary and inguinal lymph nodes, were examined in 85 cases. In 63 cases (74 per cent) the tonsils were tuberculous. In 54 (64 per cent) the upper cervical nodes on the same side as the infected tonsils were tuberculous. In 53 (63 per cent) the paratracheal nodes were tuberculous. The axillary nodes were infected in 27 (32 per cent) and the inguinal in 15

(18 per cent). If the latter figure is taken as a rough index of the hematogenous involvement of the various lymph node groups, the difference between this figure and the amount found may be taken as a rough measure of the amount of infection by lymphatic spread from neighboring tuberculous structures. It will be shown later that the incidence of hematogenous infection of the spleen and liver was much higher than the figure for the inguinal nodes, but the latter seems justifiably used for the comparison of infection in lymph nodes.

It is interesting to note that tuberculosis of the axillary nodes was detected in one-third of the cases examined. Frequently, the small tubercles found were central, suggesting hematogenous origin. About equally commonly they appeared peripheral in origin, as if the result of lymph-borne infection. In approximately two-thirds of the cases the upper cervical lymph nodes were tuberculous. If the rough measure of hematogenous infection, namely, 18 per cent, is subtracted from the incidence in the axillary nodes and the upper cervical nodes, without allowing for the number of cases that might have been infected by both routes, the latter are seen to have been involved about three times as commonly as the former. This would seem to rule out the likelihood that much if any of tuberculosis discovered in the upper cervical nodes was the result of lymphatic extension from the lungs by way of pleural adhesions.

The incidence of tuberculosis of the high paratracheal nodes was approximately the same as that found in the upper cervical nodes. Whether retrograde drainage from the former to the latter group took place in an appreciable number of cases cannot be determined. In view of the apparent relative infrequence if not actual absence of spread from the summit of the lung, however, as indicated in the preceding paragraph, spread by this retrograde route from the more remote paratracheal to the upper cervical nodes seems unlikely.

Since lymphatic and hematogenous spread of tuberculosis is believed to be more conspicuous in the Negro than in the white race, advantage was taken of the material available to test this conception. Of the 85 patients in whom the series of organs noted was examined, 26 were white and 59 were Negro. These figures are too small for accurate statistical comparison, but it is at least

of interest to note that no great difference was found in the incidence of involvement of the different organs in the two races. The inguinal nodes, believed to represent hematogenous infection chiefly, were tuberculous in 5 out of 26 cases (19 per cent) in the whites and 10 out of 59 cases (17 per cent) in the Negroes. The axillary nodes, apparently representing in their tuberculosis lymphatic and hematogenous extension about equally, were tuberculous in 10 out of 26 cases (38 per cent) in the whites and in 17 out of 59 cases (29 per cent) in the Negroes. The upper cervical nodes, probably representing chiefly spread from the tonsil and to a lesser degree hematogenous dissemination, with lymphatic spread from the lungs an unknown if existent value, were tuberculous in 18 out of 26 cases (69 per cent) in the whites and 36 out of 59 cases (61 per cent) in the Negroes.

Thus there was no evidence of a greater degree of hematogenous and lymphatic dissemination of tuberculosis in the lymph node series in Negroes than in whites.

An additional analysis was made of the part played by sex in the Negroes. As is well known, the mortality from tuberculosis is exceptionally high in young Negro women. Of the 59 Negroes, 30 were male and 29 female. The average age for males was 34 and for females 28 years. The inguinal nodes were tuberculous in 6 out of 30 cases (20 per cent) in males and 4 out of 29 cases (14 per cent) in females. The axillary nodes were tuberculous in 9 out of 30 cases (30 per cent) in males and 8 out of 29 cases (28 per cent) in females. The upper cervical nodes were tuberculous in 17 out of 30 cases (57 per cent) in males and 19 out of 29 cases (65 per cent) in females. Thus no significant difference between the sexes was noted.

CORRELATION OF TUBERCULOSIS OF THE TONSILS AND UPPER CERVICAL LYMPH NODES, AND SPLEEN, LIVER, KIDNEYS AND ADRENALS

Further analysis was made of the rôle of hematogenous infection by examining the tonsils and upper cervical lymph nodes, the organs primarily under consideration in this study, in comparison with four visceral organs, namely, the spleen, liver, kidneys and adrenals. In 102 cases all six sets of organs were studied. The tonsils were tuberculous in 76 cases (75 per cent). Upper cervi-

cal lymph nodes on the same side of the body were tuberculous
in 71 cases (70 per cent). Tuberculosis was found in 70 cases
(69 per cent) in the spleen, in 61 cases (60 per cent) in the liver,
in 23 cases (23 per cent) in one or both kidneys and in 16 cases
(16 per cent) in one or both adrenal glands.

The incidence of visceral involvement corresponds with that
commonly reported. The spleen and liver were tuberculous in
60 to 70 per cent of cases and the kidney and adrenal with far
less frequency. The spleen appears to be the most sensitive indi-
cator of the spread of tuberculosis by way of the blood stream.
It must be recalled that the figures given in this study are mini-
mal figures, in that, as a rule, only one section was examined.
Presumably the bacilli that reach the spleen enter almost exclu-
sively through the splenic artery, but they appear to find a
medium unusually suitable for growth. The liver, with a relatively
smaller arterial supply, draws blood from the portal vein and
may be tuberculous as a result of dissemination from intestinal
lesions, which were present, as noted, in about three-fourths of
the cases in this investigation. The kidneys, with a greater ar-
terial blood supply than the spleen, were much less frequently
tuberculous than the latter, although more frequently tuberculous
(23 per cent of cases) than is commonly assumed. The adrenals,
with the richest arterial blood supply of all, were discovered to
be tuberculous in but 16 per cent of cases.

In large measure the likelihood of an organ becoming tubercu-
lous is dependent upon a slow speed of circulation and the pres-
ence of phagocytic cells which may remove bacilli from the blood
stream, but fail to destroy them. The spleen and liver are vul-
nerable in these respects, even though these organs probably
destroy a large percentage of the tubercle bacilli that reach them
(Lurie [3,4]). The kidney has relatively few macrophages, but ana-
tomically is a filter with a tortuous circulation, and for this
structural reason may be expected to trap bacilli travelling in large
cells. The adrenal glands are not richly supplied with phagocytic
cells to remove bacilli, nor traplike in structure, facts probably
accounting for the low incidence of tuberculosis in this organ as
compared with the other viscera in the series here under con-
sideration.

Again, because of the common conception of excessive fre-

quency of hematogenous invasion in Negroes, a comparison was made of the incidence of tuberculosis in the several organs in the two races. At this point it should be noted that no sharp separation of miliary tuberculosis in the series studied could be made microscopically. A few of the cases were diagnosed grossly as miliary tuberculosis terminal to pulmonary tuberculosis, but many of the cases not designated as miliary grossly, exhibited extensive generalization of tubercles microscopically.

Of the 102 cases in the series, 29 were of white and 73 of Negro patients. There was approximately equal division of the sexes in the two groups. The tonsils were tuberculous in 23 out of 29 cases (79 per cent) in the whites and 52 out of 73 cases (71 per cent) in the Negroes. The upper cervical lymph nodes were tuberculous in 65 per cent of cases in whites and 70 per cent of cases in Negroes. The spleen was tuberculous in 65 per cent of cases in whites and 67 per cent in Negroes. For the liver the figures respectively for whites and Negroes were 55 and 62 per cent. For the kidneys they were 21 and 23 per cent and for the adrenals 17 and 16 per cent. Thus, by this comparison also there was no evidence that Negroes are more susceptible to hematogenous dissemination of tubercle bacilli than whites.

CORRELATION OF TUBERCULOSIS OF SPLEEN, LIVER, KIDNEYS, ADRENALS, MYOCARDIUM AND PANCREAS

A final study of hematogenous invasion was made by selecting for comparison those cases of the total 126 necropsied in which examination was made of the myocardium and pancreas as well as the spleen, liver, kidneys and adrenals. There were 96 such cases. The frequency of tuberculosis decreased in the following order: spleen, 64 cases (67 per cent); liver, 58 cases (60 per cent); kidneys, 24 cases (25 per cent); adrenals, 16 cases (17 per cent); myocardium, 2 cases (2 per cent); and pancreas, 2 cases (2 per cent).

It is perhaps noteworthy that the 4 cases in which tuberculosis was found in the myocardium or pancreas were all in Negroes. Tubercles of apparent hematogenous origin were found in the myocardium in Negro males of 17 and 24 years. The boy of 17 years had extensive generalization of tuberculosis with an old encapsulated primary lesion in a mesenteric lymph node. The

man, 24 years old, had typical gross miliary tuberculosis as a terminal process superimposed on chronic pulmonary tuberculosis. The pancreatic tubercles were found in a girl of 12 years and a man of 48 years. In each case tuberculosis was widely generalized, but not typically miliary in gross character. The tubercles noted in several instances were in the depths of the two organs. Inward extension from tuberculous pericarditis was not counted as tuberculosis of the myocardium, nor was tuberculosis of peripancreatic nodes immediately adjacent to the pancreas counted as tuberculosis of the latter.

Generalized amyloidosis and fatty infiltration of the liver were frequently present in this series. There were 8 cases of amyloidosis, or 8.3 per cent of the 96 cases, 4 of them in white patients and 4 in Negroes. The figures are too small for valid statistical comparison, but the 4 cases in whites constituted 15 per cent of the white patients and the 4 cases in Negroes only 6 per cent of the Negro cases. In all cases in which there was generalized amyloidosis the intestine was tuberculous.

Advanced fatty infiltration of the liver was noted in 25 of the 96 cases (26 per cent). The figures for tuberculosis in whites and Negroes were not significantly different; namely, 31 per cent for whites and 24 per cent for Negroes.

SUMMARY

A series of 126 cases of pulmonary tuberculosis was studied to determine the relative parts played by sputum, blood and lymph in the dissemination of tuberculosis throughout the body. The tonsils and intestine were chosen as measures of infection by sputum, and the upper cervical, paratracheal, axillary, mesenteric and inguinal lymph nodes as measures of lymphatic spread, with recognition of the fact that all of the organs named may be infected hematogenously. Hematogenous spread was studied in the spleen, liver, kidneys, adrenals, myocardium and pancreas.

Figures are given showing the frequency of infection in the several organs named. A high positive correlation existed in tuberculosis of the tonsils, upper cervical lymph nodes, intestine and mesenteric lymph nodes, presumably indicating sputum infection of the organs of the alimentary tract and secondary involvement of the draining lymph nodes by the lymph stream. Of the

several series of lymph nodes examined, the superficial inguinal nodes were infected the least frequently. Their incidence of tuberculosis was considered a rough measure of the extent of hematogenous spread to the lymph nodes, which could be subtracted from the total incidence of infection of the other lymph nodes to furnish a rough minimal index of the involvement of the latter by way of the lymph stream.

The tonsils and intestine were tuberculous in about three-fourths of the cases, and the upper cervical and mesenteric lymph nodes in almost three-fourths. Tubercles were found in the axillary nodes in one-third and in the inguinal nodes in more than one-sixth of the cases of the series.

A striking difference in the incidence of hematogenous infection was apparent on examination of the viscera. Single section examination showed tubercles in approximately two-thirds of the cases in the liver and spleen, in one-fourth in the kidney, 16 per cent in the adrenal and 2 per cent each in the myocardium and pancreas.

Although tuberculosis is commonly believed to exhibit a greater tendency to hematogenous and lymphatic spread in Negroes than in whites, no significant difference between the races was found in these respects in the series here reported, nor was any difference found between the sexes.

The incidence of generalized amyloidosis was 8 per cent and of advanced fatty infiltration of the liver, 26 per cent. No racial difference was apparent.

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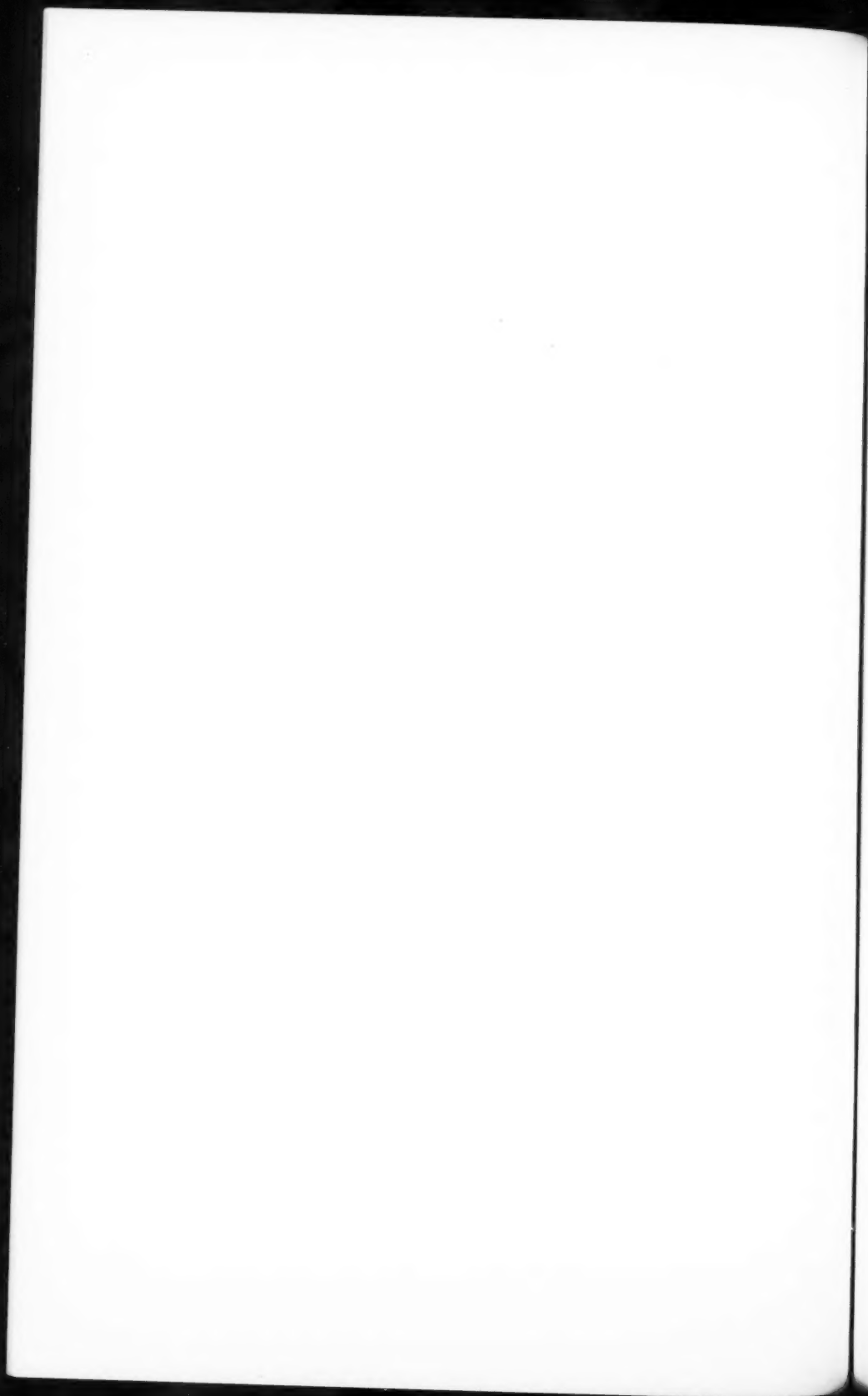
CAVITIES IN THE SILICOTIC LUNG*

A PATHOLOGICAL STUDY WITH CLINICAL CORRELATION

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The presence of a cavity in any lung has a serious clinical import, not only because it represents destruction of pulmonary tissue, but because it serves as a source of numerous complications. In the silicotic lung more cavities are observed at the autopsy table than are recognized during life by X-ray or physical examination. Pathological investigation has shown that all of these cavities are not alike and it is possible to differentiate three distinct types:

1. Cavities associated with a *typical tuberculosis* that is little modified by coexistent silicosis.

2. Cavities occurring in areas of *tuberculo-silicosis*, an extremely chronic condition resulting from the combined local effect of tubercle bacilli and silica dust. In them, the results of infection are obvious and tubercle bacilli are usually demonstrable.

3. Cavities of the so-called *anemic type* which develop within areas of massive fibrosis, but show no evidence of causative organisms or cellular reaction.

TYPICAL TUBERCULOUS CAVITIES

Cavities closely resembling those found in uncomplicated tuberculosis (Fig. 1) usually occur in cases with minimal silicotic nodulation. Such cavities may be large or small, unilocular and spheroidal or multilocular with numerous extensions into the surrounding lung. They usually contain a thick purulent liquid which varies in amount depending upon the patency of the draining bronchus. A wall of inflammatory tissue demarcates them from the air-containing lung. The structure of their wall varies with the chronicity of the lesion. In the rapidly progressing types it is soft and pliable, whereas in those that develop more slowly and have existed for a long time the wall is usually firm and rigid.

Microscopically, three definite zones can be identified in the wall (Fig. 2). The inner lining of the cavity is a soft gray, pyogenic membrane. Beneath this membrane is a zone of granulation tissue with numerous dilated capillaries, many lymphocytes and large mononuclear cells which in the chronic forms are replaced by fibrous tissue. The latter is never as thick nor as dense as the mature hyaline tissue about cavities of tuberculo-silicotic origin (Fig. 4). Occasional silicotic nodules, incorporated in the wall, may be partially eroded by the ulcerative process or they may exhibit a caseous center surrounded by a layer of inflammatory cells. The concentric laminae of thick hyaline fibers distinguish these nodules from the more irregular tubercles that usually occur in the immediate vicinity. For details of differentiation reference is made to the recent publication by Gardner.[1]

Tubercle bacilli can be easily demonstrated in the contents or wall of these excavations. Their tinctorial and cultural characteristics and their virulence for guinea pigs are similar to those of bacilli of human type recovered from lesions of uncomplicated pulmonary tuberculosis.

The origin and development of such cavities are essentially the same as those of uncomplicated pulmonary tuberculosis. Although the process may be accelerated by the presence of silica in the lung, the pathology and clinical manifestations of the infectious element remain unaltered. Either the amount of silicosis or the state of activity seems to have been insufficient to modify the course of the associated infection.

TUBERCULO-SILICOTIC CAVITIES

Excavations of this type (Fig. 3) are invariably found within areas of massive, hard, black to gray conglomerate fibrosis. Most of them are small and unilocular. The thick, dense fibrous tissue surrounding them apparently resists necrotizing action and prevents the extension productive of multilocular forms. The content of these cavities is purulent, but inky-black. Occasionally small gray-black masses of silicotic fibrous tissue are found free in the pus. Some of them represent sequestrated nodules eroded from the wall in the process of tissue degeneration. The trabeculation, so common in the uncomplicated tuberculous cavity, is seldom seen, probably because the blood vessels are no more

resistant to the very slow process of necrotization than the very dense fibrous tissue that surrounds them.

The most important feature of these cavities is the zone of dense black fibrous tissue which surrounds them. The usual internal necrotic membrane (Fig. 4) is extremely thin and may line only a portion of the cavity. It often blends imperceptibly with the overlying pigmented scar tissue and does not exhibit the pronounced pyosis so common in simple tuberculous excavations. It is probable that in the early stages of such cavities there may be considerable infiltration with leukocytes, but as they become older the avascularity which is concomitant with massive fibrosis retards migration of such cells from the blood stream. As a consequence the lining membrane simulates that observed in simple necrosis rather than in tuberculosis.

A conspicuous feature is the absence of an intermediate zone of hyperemic granulation tissue, which is so characteristic of simple ulcerative phthisis. Although the microscope may reveal granulations in an occasional minute portion of the wall, such areas are few and poorly vascularized.

The zone of conglomerate fibrosis surrounding the cavity may be only 2 to 3 cm. in thickness, but more often it involves a large part of a lobe and may even cross the fissure into the neighboring lobe. With extension to the periphery of the lung, the pleura is thickened and firmly attached to the adjacent chest wall by focal adhesions or an obliterating fibrous pleurisy.

The tuberculous components of the conglomerate lesion, apart from the cavity, may be difficult to identify since they are modified by the tissue response to the particles of silica. The foci of caseation scattered throughout the mass are generally smaller and firmer than in ordinary tuberculosis. Microscopically, there are usually areas in which hyaline bands of fibrous tissue are poorly stained and appear swollen and hazy in outline. In and about such areas there is often an infiltration of inflammatory cells, but these are rarely concentrated in foci to form tubercles.

The histopathological characteristics of the complicating tuberculosis are so atypical that in some instances bacteriological methods must be employed for diagnosis. Acid-fast bacilli are so sparsely scattered throughout the massive wall of the cavity that often they cannot be found on direct examination. Guinea

pig inoculation or culture, however, usually demonstrates their presence.

Other features of the massive wall about the tuberculo-silicotic cavity are also significant. The hyaline, nodular components of the lesion can usually be identified in regions least involved by the tuberculous process. In some of the mature reactions, however, even this feature may be obscured since the contraction of the heavily pigmented matrix draws the nodules close together and distorts their boundaries, ordinarily clearly defined.

The pigmentation is due to the particles of nonsiliceous minerals that were inhaled with the free silica in the working atmosphere. In the case of coal this consists of black carbonaceous material; the associated oxides of iron, magnetite and hematite, respectively cause a black or red pigmentation. More attention should be given to this nonsiliceous component of the inhaled dust because of its effect upon the inflammatory response to the particles of silica. Experimental studies have shown that coal and these oxides of iron retard and modify the usual reaction to silica in the pure form.[2] The mechanism of this inhibitory action has not been completely established.

In an area of massive fibrosis practically all the normal pulmonary structures are involved. The blood vessels reveal an advanced obliterative arteritis and phlebitis. The bronchi and bronchioles are markedly compressed and distorted by the surrounding fibrosis and by the inflammatory changes within their walls. The epithelial lining is frequently desquamated, but where present it shows no evident hyperplasia.[3] The submucosa is thickened by granulation tissue and occasionally contains small proliferative tubercles.

The intrapulmonary and extrapulmonary complications of ordinary tuberculous cavities rarely accompany excavations of the tuberculo-silicotic type. Hemorrhage may occur as a late manifestation, but usually the avascularity of the wall prevents the repeated hemoptyses so common in uncomplicated ulcerative phthisis. The same factor undoubtedly retards hematogenous dissemination of bacilli, for miliary tuberculosis is uncommon. Spread by aspiration throughout the lungs, and also tracheobronchial, laryngeal and intestinal complications, are all rare.

The evolution of the tuberculo-silicotic cavity in the living sub-

ject is hard to follow. The large masses of fibrous tissue, in which such cavities develop, form very slowly. Such lesions are so dense that they are not readily penetrated by the X-ray, with the result that even the specific evidences of tuberculosis are hard to recognize. Because of the delayed appearance of a positive sputum and of symptoms referable to tuberculosis, no warning is given of changes that may be taking place within areas of massive fibrosis. Progression of a primary infection to the stage of cavity formation is conceivable. The Saranac Laboratory museum has one case in which this mechanism seems probable. Ordinarily, however, the pulmonary portion of the primary complex of childhood no longer contains living organisms by the time dust exposures begin. Primary infections acquired later, after silicosis has developed, tend to run an acute course resulting in the so-called "perinodular tuberculosis." Such cases terminate in tuberculous pneumonia which may be complicated by rapid formation of cavities, but these lesions are outside the scope of this paper. The ordinary chronic cavity of tuberculo-silicosis is probably the result of a reinfection whose source may be either exogenous or endogenous. If of the latter origin it may result from the reactivation of a latent encapsulated focus of infection.

The resistance of the host and the number and virulence of the infecting organisms play a part, but one of the chief factors is an active silicotic lesion. In experimental lesions which are in a stage of necrosis, tubercle bacilli multiply with unusual rapidity.[4] In animals it has been shown also that latent tuberculous lesions, which in normal hosts tend to heal and disappear, will become reactivated and spread under the influence of subsequently inhaled silica.[5] These results follow without reference to the native or artificially induced resistance in the host.[6]

ANEMIC CAVITIES

As in tuberculo-silicosis, cavities of anemic type (Fig. 5) also are found within pigmented areas of conglomerate fibrosis. They are differentiated from those in tuberculo-silicosis by the following characteristics. The cavities are usually small, elongated and slitlike. In a few cases, however, they have been of considerable size, occupying a large portion of a lobe. Their content is inklike,

but as compared with the exudate in tuberculous cavities it is more fluid and not purulent. The well defined inner zone of purulent material that lines tuberculous cavities is absent. In its place is an indefinite, ragged zone of seminecrotic tissue of the same color as the surrounding fibrosis. There are no trabeculae. The thick limiting wall of black fibrous tissue shows nothing suggestive of tuberculosis.

Microscopic sections (Fig. 6) reveal ends of fibrous strands terminating abruptly at the lumen of the cavity. Often small degenerated fragments hang into the cavity and because of their indefinite structure and poor staining quality they appear as ghost strands. The dust cells usually found between the fibers are disintegrated and their particles lie scattered throughout the area. More distant from the cavity, the massive wall is uniformly a heavily pigmented fibrous tissue of the type commonly seen in anthracosilicosis or in reactions to other mixtures of free silica with various minerals.

The blood vessels within the mass show arteritis and phlebitis of a high grade. The earliest changes recognizable in the arteries are a thickening of the intima and a narrowing of the lumen by a new formation of connective tissue. With further advance of the disease the whole structure is transformed into a dense connective tissue containing scattered dust particles. At this stage the vessel can no longer be differentiated from the surrounding fibrosis except perhaps by elastic tissue stains. The veins are also involved, but in the more chronic lesions they cannot be identified.

The remarkable feature about these excavations, setting them apart from the tuberculous cavities, is the absence of histopathologic and bacteriologic evidences of infection. Capillary dilatation, edema and leukocytic infiltration with the formation of an inner pyogenic membrane are absent. Tubercle bacilli cannot be demonstrated either by staining or animal inoculation.

These cavities result from a combination of factors, of which the most important appears to be vascular. Both roentgenographic studies of the lungs after injection of radio-opaque material into the vascular tree and histological studies demonstrate that the blood vessels in the massive fibrotic areas are practically obliterated. The resulting anemia in the depth of the lesion is fol-

lowed by areas of local necrosis which because of autolysis ultimately liquefy and excavate.

Other possible factors which must be considered in the etiology of such necrosis and excavation are the toxic action of silica and the modifying influence of the nonsiliceous dust that usually contaminates the lesion. Both clinical and experimental evidence indicates that silica in high concentration is toxic and kills tissue. The nonsiliceous dusts may be concentrated in such areas simply because the fibrosis has occurred, or it is possible that the heavily pigmented tissue is actually more sensitive to a deficient blood supply. As a matter of observation, necrosis, liquefaction and resultant excavation begin in pigmented scar tissue and ultimately involve all of the structures in the immediate vicinity.

LOCATION OF CAVITIES

Table I compares the location of the cavities in 339 cases of uncomplicated tuberculosis with those in 94 cases of silicosis. The latter are subdivided into typical tuberculous cavities in silicotic lungs (34 cases) and tuberculo-silicosis (60 cases).

It will be noted that in all three types of disease cavities may occur in any part of the lung, but in all they are most frequent in

TABLE I

Comparison of Location of Cavities in 339 Cases According to Type

Lobe of lung	Simple tuberculous cavities	Typical tuberculous cavities in silicotic lungs	Tuberculo-silicotic cavities
	(*per cent*)	(*per cent*)	(*per cent*)
Upper	76	67	62
Middle	7	9	8
Lower	17	24	30

the upper lobes. In the lower lobes the incidence is nearly twice as great in the tuberculo-silicotic cases as in those with uncomplicated tuberculosis. The frequency of middle lobe involvement is approximately the same in all three categories. A point of difference, not indicated in the table, is a predilection toward localization in the midportion and lower portion of each lobe of the silicotic lung. In nonsilicotic subjects more cavities occur in the posterior portions of the lobes.

CLINICAL CORRELATION

The *typical tuberculous cavities* are in no way unusual in the subject with early discrete nodulation. The clinical picture is the same as that in simple chronic phthisis and the lesions are associated with the common pulmonary and extrapulmonary complications. The clinical problem in these cases is primarily one of diagnosing the concomitant silicosis. The X-ray is the most reliable means of differentiating silicotic nodules from tubercles of bronchogenic or hematogenous origin. In cases with advanced infection differentiation may be extremely difficult or impossible.

In the tuberculo-silicotic subject the clinical problem is more difficult. The modifying effect of the silicosis upon the tuberculous element in this disease alters the picture so that the symptoms exhibited are characteristic of neither of the conditions in uncomplicated form. For this reason the condition of *tuberculosilicosis* must be regarded as a separate disease entity.

Frequently it requires an over-exposed film to demonstrate the presence of a cavity in the center of an area of massive fibrosis. The characteristic signs and symptoms usually accompanying excavations in chronic phthisis are often absent. The peculiarities of the pathology of tuberculo-silicosis with cavity formation will explain this apparent paradox. The pronounced avascularity not only of the fibrous tissue immediately about the excavation, but also throughout the surrounding thick zone of hyalinized scar, retards the absorption of toxic products elaborated at the site of the cavity. As a result, these patients seldom experience the marked loss of weight, the weakness and the elevated temperature, or other toxic manifestations of uncomplicated pulmonary tuberculosis. The absence of the hyperemic zone in the cavity wall also explains the rarity of other complications, such as hemorrhage and hematogenous spread of disease, that commonly accompany simple tuberculous cavities. The compression and occlusion of the air passages by a mass of rigid scar tissue retard the drainage from the cavity so that the sputum often remains negative for tubercle bacilli until late in the course of the disease. In a few cases repeated examinations have failed to demonstrate bacilli throughout life although after death they were found in the wall of the cavity.

Because of these peculiarities, cases of tuberculo-silicosis seldom develop early spread of the disease either by contiguous extension or through the bronchial tree. Secondary complications such as tuberculous tracheobronchitis, laryngitis and enteritis are much less frequent than in ordinary tuberculosis.

The *anemic cavities* offer very few clinical features for correlation. Usually the excavations are so small that they cannot be differentiated from the surrounding mass of conglomerate fibrosis either by roentgenographic methods or other means at the disposal of the examining physician. When demonstrated, they are most often interpreted as tuberculous, since their location is not unlike that of the tuberculo-silicotic cavities.

SUMMARY AND CONCLUSIONS

This paper summarizes the pathologic features and associated clinical picture of three types of cavities commonly encountered in the silicotic lung.

Typical tuberculous cavity formation develops most often in persons with minimal silicosis. It is characterized by the ordinary pathological and clinical features of excavation in uncomplicated chronic phthisis.

In the massive fibrotic lesions of tuberculo-silicosis, produced by the combined local action of silica and tubercle bacilli, cavities of modified form usually develop. They differ in morphology from the simple tuberculous cavities and do not give rise to the usual symptoms until the disease has existed for a very long period.

Anemic cavities also may occur within massive lesions of conglomerate fibrosis without evidence of active infection. They are probably due to local deficiency in blood supply, although the toxic action of high concentrations of silica may play a part. Of themselves, they do not cause recognizable clinical symptoms.

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DESCRIPTION OF PLATE

PLATE 117

FIG. 1. Tuberculous cavity in a silicotic lung. The wall is thin and contains
scattered, deeply pigmented, silicotic nodules. × 0.85.

FIG. 2. The wall of the tuberculous cavity showing a well developed pyo-
genic membrane, capillary lumina in the granulation tissue zone and a
peripheral zone of non-silicotic fibrous tissue. × 45.

FIG. 3. Tuberculo-silicotic cavity occupying the middle lobe. The interlobar
fissures are obliterated. The component nodules of the massive con-
glomerate lesion are easily identified. × 0.4.

FIG. 4. The wall about the tuberculo-silicotic cavity. The pyogenic mem-
brane is thin and blends imperceptibly with the hyalinized fibrous tissue
responding to silica. An intermediate zone of hyperemic granulation
tissue is absent. × 45.

FIG. 5. Anemic cavity within a massive conglomerate lesion of silicotic
fibrous tissue. The scar obliterates the fissure and involves a portion of
the lower lobe. × 0.4.

FIG. 6. The wall immediately about the anemic cavity. It is lined by an
indefinite ragged zone of seminecrotic tissue. A pyogenic membrane is
absent. × 45.
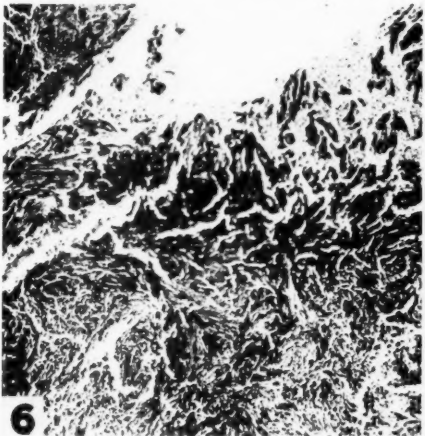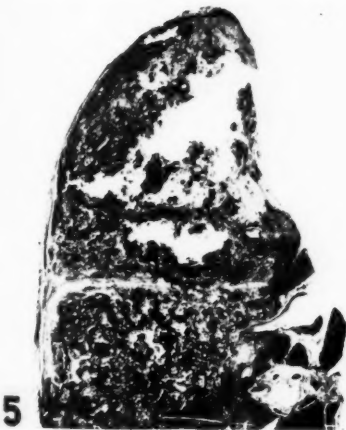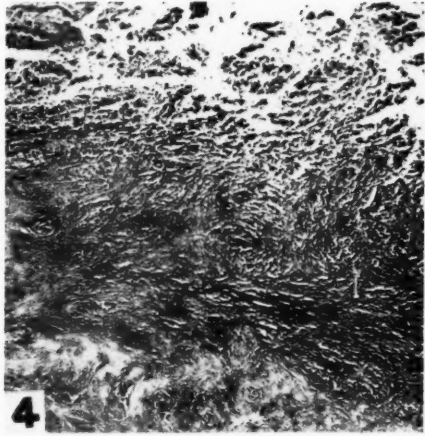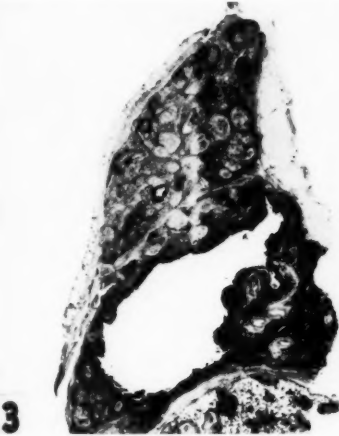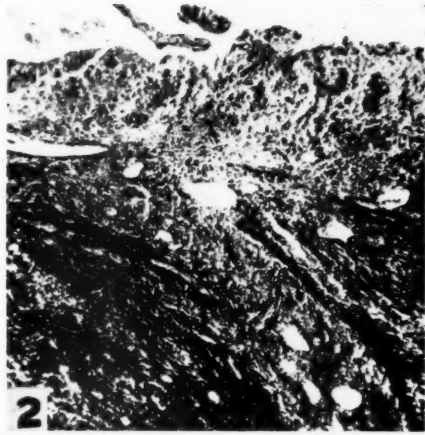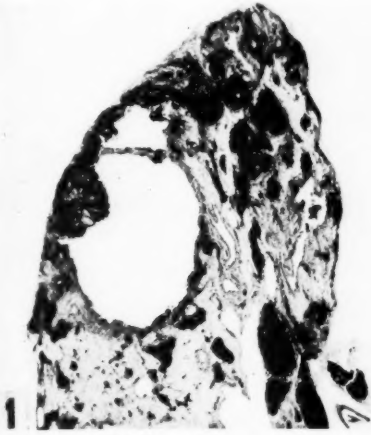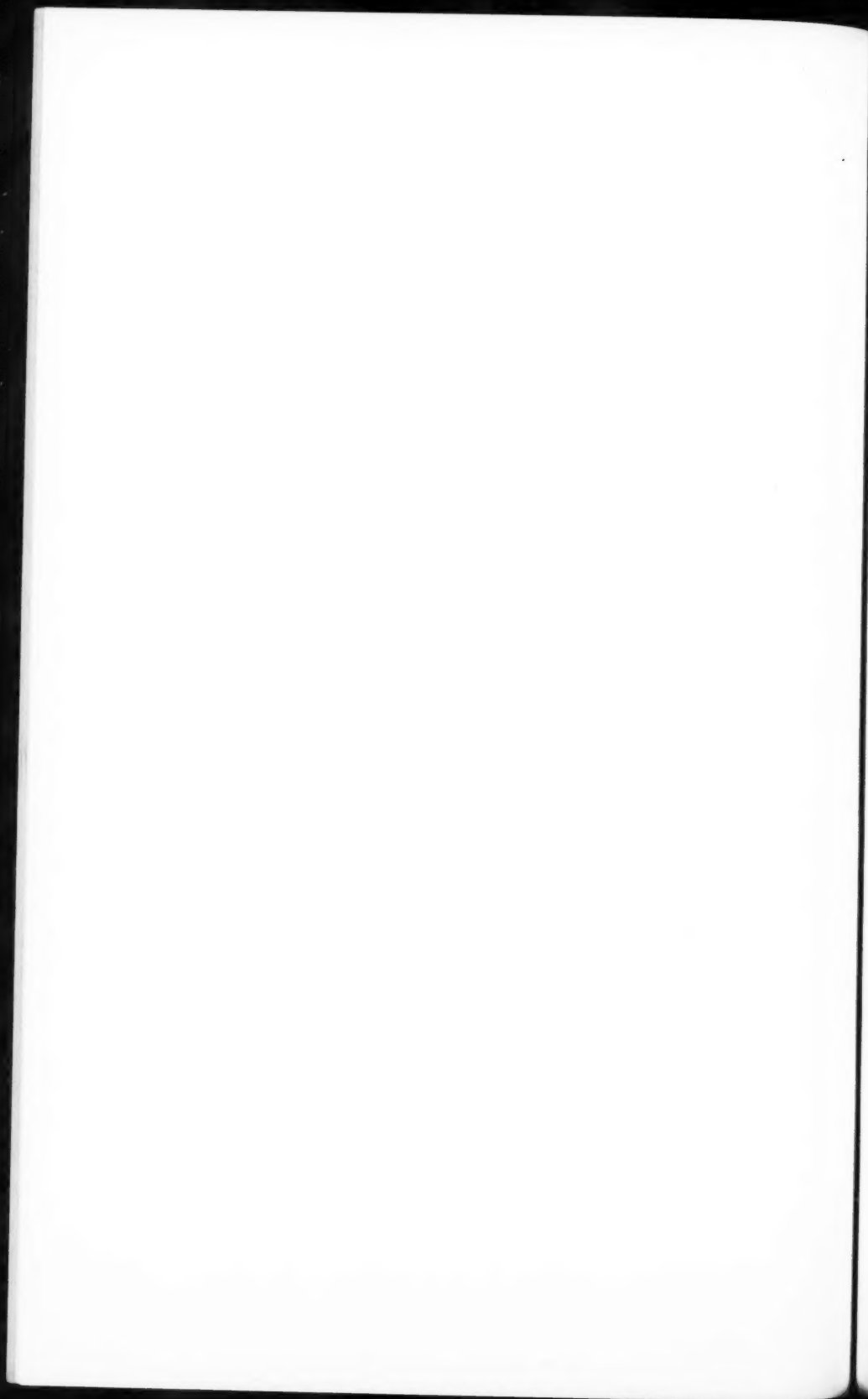
THE AGE FACTOR IN ACTIVE IMMUNIZATION AGAINST WHOOPING COUGH*

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Because whooping cough is fatal only during the first 2 years of life, and since it causes more infant deaths than do the other preventable contagious diseases—diphtheria, scarlet fever and smallpox—active immunization, if it is to be effective, should be attempted as early as immunity can be conferred. The vaccine should not elicit severe local or systemic reactions; nor should it sensitize the individual. Authorized† *Haemophilus pertussis* vaccine referred to in this report was made with human blood; it was not "washed" because it contained no animal protein.

Two opportunities presented themselves for obtaining controlled data on the use of authorized *H. pertussis* vaccine as an immunizing agent in the first months of life. One was at the Evanston "Cradle," a placement shelter for newborn infants; the other was at the four infant welfare stations of the Chicago Health Department where the highest incidence of deaths from pertussis had occurred.

In 1933, the director of the "Cradle" requested the injection of each thriving infant more than 1 month of age and weighing more than 7 lbs. Although it was suggested at the time that if alternate infants were injected, the uninjected would serve as controls, controls from another source were established instead. Authorized *H. pertussis* vaccine was used. At first, each infant was injected with 1 cc. weekly in alternate arms for 6 successive weeks, so that a total of 60,000 million bacilli was injected. Not until 1934 was the customary total dosage of 80,000 million bacilli given to these young infants. About 200 "Cradle" infants less than 3 months of age were injected each year for 7 years. These very young infants withstood the injections remarkably well. The rectal temperature seldom rose above 38.5° C.; the local reaction was usually transient. A subcutaneous nodule

formed, but seldom persisted for more than a fortnight. Never did a local abscess occur. On January 1, 1940, the director mailed a questionnaire to the foster parents of 1,434 injected children. From these 1,110 replies were received. The average age of the children at that time was about 4 years. Of the 91 who had had pertussis, 68 were in families having no other children.

For controls, Dr. James P. Campbell, of the Geneva Community Hospital, compiled data on the attack rate of pertussis in children born in the obstetrical department between 1933 and 1939. In this hospital, which is located 45 miles from Chicago, somewhat fewer than 200 infants are born annually. On January 1, 1940, the superintendent mailed a questionnaire to the parents of 1,000 living children born there during the previous 7 years. From these 464 replies were received. The average age of the children at the time of the investigation was somewhat less than 4 years. Forty-two were in families having no other children. Vaccine had not been given to 256; 208 had had authorized *H. pertussis* vaccine after the age of 7 months. No Geneva child injected since 1935 has developed whooping cough. In Table I the attack rates of the control group (no vaccine) and the "Cradle" group are compared with the attack rates of three groups injected after the sixth month. The attack rate of the injected Geneva group coincides with the attack rate of the Evanston Health Department clinic group and is lower than that of Evanston privately injected older infants.

Before the end of 1934 and in each year since then, children injected with the vaccine made in 1934 have developed the disease. Many of these cases can be attributed to the impotency

TABLE I
Age Factor in Active Immunization Against Pertussis

Group	Period	Age when injected	Number of infants	Had whooping cough	Attack rate
		months			*(per cent)*
Geneva (controls—no vaccine)	1933–39		256	38	14.84
Cradle	1933–39	2 to 3	1110	91	8.19
Geneva private	1933–39	over 7	208	3	1.44
Evanston clinic	1934–39	over 8	1586	20	1.26
Evanston private	1933–39	6 to 8	1206	30	2.48

of the vaccine made in that year, for not until 1935 was a gross error in vaccine production discovered and rectified. Text-Figure 1 shows the attack rate arranged according to the years in which the "Cradle" infants (A) and the older infants (B) were injected. Although the attack rate of each group decreased after 1935, that of the "Cradle" group remained the higher of the two. Of the 91 "Cradle" infants who later developed pertussis, 34 had
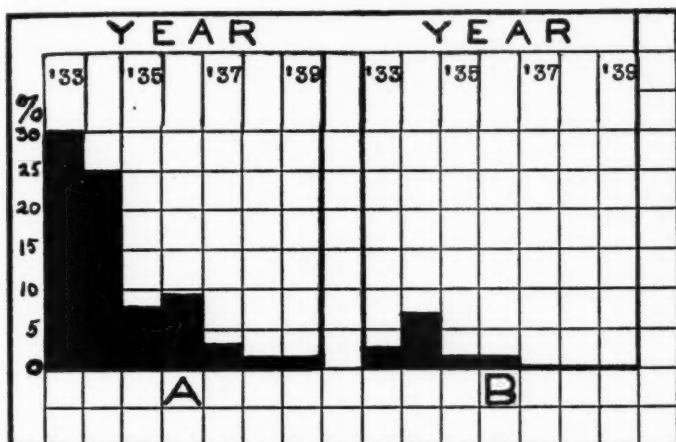
TEXT-FIGURE 1. Percentage of pertussis (1933 to January 1, 1940). A, injected before 3 months; B, injected after 7 months.

had 1934 vaccine; of the 3 privately injected Geneva infants who contracted the disease, 2 had had 1934 vaccine; of the 20 Evanston clinic infants, 17 had had 1934 vaccine; and of the 30 privately injected Evanston infants, 22 had had 1934 vaccine. Of the children injected after the seventh month who later developed pertussis, over 85 per cent were injected with 1934 vaccine. No serious complication and no death from whooping cough has been reported in any injected child who subsequently developed the disease.

On 89 "Cradle" infants 123 complement-fixation tests were performed* from 1 to 12 weeks after the final injection of vac-

* At first by Mr. F. G. Jones of Eli Lilly Laboratory; more recently by Mrs. Eva Markley of the Evanston Hospital Whooping Cough Research Laboratory.

cine. Of these tests 65 were negative; 10 were $+$; 13 were $++$; and 1 was $+++$. Similar tests and retests on older infants, performed from 1 to 12 weeks after the final vaccine injection, were usually positive; in over 70 per cent the test was $+++$ or $++++$.

The intracutaneous pertussis test was performed on 35 "Cradle" infants from 1 to several weeks after the final vaccine injection. One part of the clear supernatant fluid (after prolonged centrifugation of 15,000 million bacilli per cc. authorized vaccine) was diluted with fifteen parts of 0.5 per cent phenol in 0.85 per cent sterile saline. When 0.1 cc. was injected intracutaneously, the test was negative when observed 24 hours later. When performed in older infants, from 1 to 12 weeks after the final vaccine injection, an erythematous area at least 10 mm. in diameter was usually observed 24 hours later.

The other opportunity for observing the effect of authorized *H. pertussis* vaccine injections in young infants occurred in 1936 when Dr. Herman N. Bundesen, Chicago Commissioner of Health, requested the injection of alternate infants between the second and sixth months of life at the four welfare stations where the highest mortality from pertussis had occurred. During that summer 1,071 infants were injected. One year later, the parents of 758 were located; the remainder had moved without leaving an address. Twelve of these infants had developed whooping cough and 8 had been exposed and escaped. Of the 998 unvaccinated control infants in the same welfare stations, the parents of 757 were located. Eleven of these infants had developed whooping cough and 2 had been exposed without developing the disease.

From Table II it will be seen that the attack rate was not reduced by the injection of vaccine before the sixth month of

TABLE II
Data from Four Chicago Health Department Welfare Stations

Group	Number of infants	Number located 1 year later	Had whooping cough within the year	Attack rate (per cent)
Controls (no vaccine)	998	757	11	1.45
Total dosage 8 cc. authorized *H. Pertussis* vaccine	1071	758	12	1.58

life. The attack rate of 1.58 per cent at the end of 1 year was as high as that at the end of 7 years for all groups injected after the seventh month of life. Furthermore, the attack rate in the welfare clinic group, injected between the second and sixth months, was as high as that of the "Cradle" group, injected between the second and third months.

DISCUSSION

Although some infants at the age of 3 months possess the power to elaborate the specific antibody from hypodermically injected authorized *H. pertussis* vaccine, others seem to lack this power. Most of the "Cradle" infants injected after 1934, who developed pertussis later, had it in a mild form. No death from whooping cough has been recorded to date in any child who developed the disease after injection with authorized *H. pertussis* vaccine.

SUMMARY

Pertussis developed in 14.84 per cent of the Geneva control children who had had no vaccine; in 8.19 per cent of the "Cradle" children injected before the third month of life; in 2.48 per cent of the Evanston private patients injected after the sixth month of life; and in 1.44 per cent of the Geneva children injected after the seventh month of life.

CONCLUSIONS

1. Pertussis occurred seven times more frequently in children injected before the third month of life than in children injected after the seventh month.

2. For active immunization authorized *H. pertussis* vaccine should be injected after the seventh month of life.
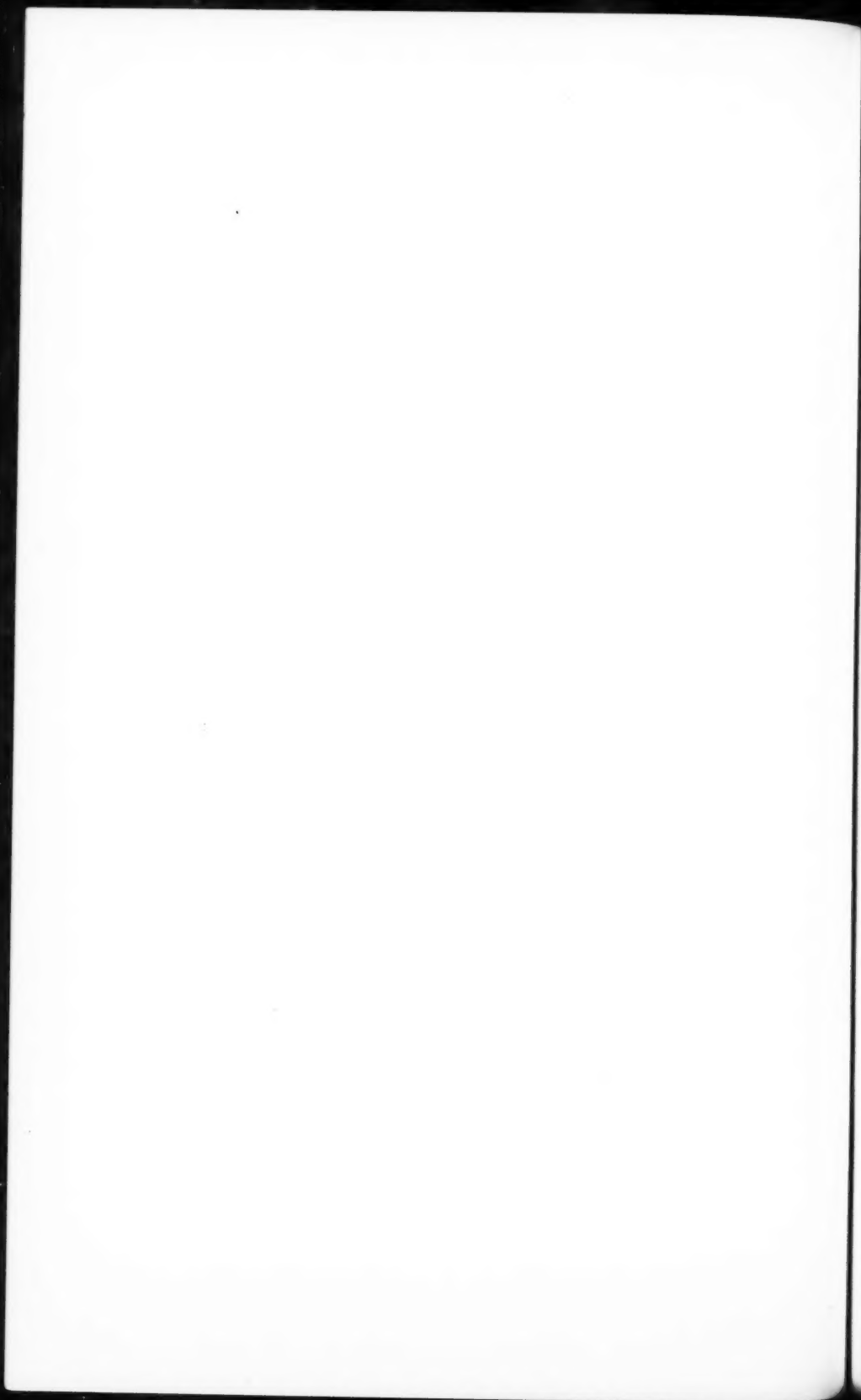
THE ANTIGENIC RELATIONSHIP OF ALCOHOL-SOLUBLE SUBSTANCES OF CORPUS LUTEUM TO THOSE OF TESTIS AND BRAIN*

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In a previous communication[1] it was shown that alcohol-soluble substances from the testis have antigenic properties very closely related to those from brain. An antiserum for lipoids of beef brain will give complement-fixation reactions with lipoids of beef testis and an antiserum for beef testis will react with lipoids of beef brain. Carefully conducted quantitative tests show that, with complement-fixation reactions at least, the alcohol-soluble antigens of these two organs are indistinguishable.

Not only have beef brain and testis an antigenic reactivity in common with each other but also in common with brain and testis of all other species tested, including the rabbit which was the source of the antiserums used in the experiments. These organs, therefore, lack species specificity but have a specificity that is presumably based on some common constituent present in brain and testis of various species. These facts fulfilled the conditions for iso-antigenicity, a property that has been demonstrated for brain[2] but not as yet for the testis, although there is no reason to believe that the experiments demonstrating iso-antigenicity for brain will give different results when performed with the antigenically related testis.

Since the ovary is the female homologue of the testis, the question arises as to whether the alcohol-soluble substances of the female organ share the antigenic specificity of the male organ. The present report concerns this question and deals with complement-fixation reactions between anti-brain, anti-testis and anti-corpus luteum serums and homologous and heterologous organ extracts.

The material used to prepare ovarian alcoholic extracts was a large number of fresh glands obtained from slaughtered beef. The ovaries were washed free of blood and the small amount of adventitial tissue trimmed off. Each gland contained one or

more bright yellow corpora lutea, ranging in size from 1 or 2 mm. to about 1.5 or 2.0 cm. in diameter. These could be shelled out easily, the operation effecting a fairly sharp separation. The greatest difficulty was experienced with the small corpora lutea in that they were not easy to find or to separate cleanly. Many of these were revealed and made accessible for removal by cutting the ovary into thin slices.

The corpora lutea and the remaining ovarian tissue were separately ground in a meat grinder, placed in bottles containing about ten volumes of 95 per cent alcohol, and kept in the incubator 10 to 14 days. After this period the alcoholic solutions were filtered off while warm. On cooling to room temperature a white precipitate formed, most of which could be redissolved by adding alcohol. The extract of the corpora lutea was clear, deep yellow and contained 0.925 per cent solids. That of the ovary was a clear, straw-colored solution containing 0.714 per cent solids. Alcoholic extracts of other organs used in these experiments were prepared in a similar manner.

For the preparation of antigens for complement-fixation tests, measured quantities of the extracts containing known amounts of solids were evaporated to dryness. The residues were triturated in saline and made up to such a volume that 0.5 cc. contained 1 mg. of lipoid. From this stock emulsion, dilutions were made. It has been found that equally active antigens can be prepared by adding measured quantities of the alcoholic extracts directly to saline. Such preparations should be boiled to remove most of the alcohol, the volume lost by evaporation being replaced with distilled water. The relative volumes of extract and saline used are chosen to give the proper concentration of lipoids.

The antiserums of brain and testis used in these experiments were obtained from rabbits given repeated intravenous injections of suspensions in saline of the finely ground fresh organs. Anticorpus luteum serums were from rabbits injected repeatedly and intravenously with an emulsion of the lipoid from beef corpora lutea mixed with horse serum.

The results of complement-fixation reactions of antiserums for beef brain, testis and corpus luteum with alcoholic extracts of various beef organs indicate the antigenic similarities and differences among the lipoids of the organs (Table I). No reactions

TABLE I

Reactions of Alcoholic Extracts of Beef Organs with Antiserums for Beef Brain, Testis and Corpus Luteum.

Beef organ extracts	Beef organ antiserums	Milligrams of alcoholic extract residue									
		1.0	0.6	0.4	0.2	0.1	0.06	0.04	0.02	0.01	0.006
Corpus luteum	Corpus luteum	o o	o o	o o	o o	o o	o o	o o	+ o +	+ + / + +	+++ +++ +++ +++
	Testis	o o	o o	o o	o o	o o	o o	o o	+ o o	+ + o	+++ +++ + +++
	Brain									+ +	+ + + / + + + / + + + / +
Testis	Corpus luteum	o o	o o	o o	o o	o o	o o	o o	+ o o	+ + o	+++ +++ +++ ++
	Testis	o o	o o	o o	o o	o o	o o	o o	+ o o	+ + o	+++ +++ +++ +
	Brain										+ + + / + + + / +
Brain	Corpus luteum	o o	o o	o o	o o	o o	o o	o o	+ o o	+ +	+ + + / + + + / + + + / +
	Testis	o o	o o	o o	o o	o o	o o	o o	+ o o	+ +	+++ +++ +++ +++
	Brain										
Ovary	Corpus luteum	+	o o	o o	o o	o o	o o	o o	+ o +	+ +	+++ +++ +++ +++
	Testis	++++	++++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Brain	+	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
Liver	Corpus luteum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Testis	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Brain	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
Kidney	Corpus luteum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Testis	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Brain	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
Heart	Corpus luteum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Testis	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Brain	+	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
Lung	Corpus luteum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Testis	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Brain	+	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
Spleen	Corpus luteum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Testis	++++	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++
	Brain	+	+++	+++	+++	+++	+++	+++	+++	+++	+++ +++ +++ +++

The details of the technic used in performing the complement-fixation tests are described in a former article.[4]
o = absence of hemolysis and complete fixation of complement; ++++ = complete hemolysis and no fixation of complement; +, ++, +++, = various degrees of hemolysis and fixation of complement between o and ++++.

728 LEWIS

with any of the antiserums were obtained with extracts of liver, kidney, heart, lung or spleen. The extract of ovary from which corpus luteum tissue was removed gave either no reaction or weak reactions. In contrast, the extracts of brain, testis and corpus luteum gave strong reactions with the homologous as well as with heterologous antiserums. It is possible that the weak reactions of the ovarian extract were due to an incomplete removal of corpora lutea from the ovaries in the preparation of the extract.

It is thus seen that brain lipoids, believed at first to be markedly organ-specific,[3] have a common reactivity with those of at least two other organs. The possibility also exists that comparisons with the lipoids of other organs not included in these experiments, such as the adrenal, pituitary and thyroid, will disclose still other antigens that have antigenic properties in common with brain lipoids.

Another similarity of corpus luteum lipoid to lipoid of brain and testis is its lack of species specificity as indicated by the positive reactions obtained between an anti-human brain serum and beef corpus luteum lipoid, and between anti-beef corpus luteum serum and sheep brain lipoid (Table II).

TABLE II

Reaction of Beef Corpus Luteum Extract with a Heterologous Brain Antiserum and of Beef Corpus Luteum Antiserum with a Heterologous Brain Extract

Extract of	Antiserum for	Milligrams of alcoholic extract residue									
		1.0	0.6	0.4	0.2	0.1	0.06	0.04	0.02	0.01	0.006
Beef corpus luteum	Human brain	o	o	o	o	o	o	+	+ +	+ + +	+ + + +
Sheep brain	Beef corpus luteum	o	o	o	o	o	o	o	+ +	+ + +	+ + + +

o = absence of hemolysis and complete fixation of complement; + + + + = complete hemolysis and no fixation of complement; +, + +, + + + = various degrees of hemolysis and fixation of complement between o and + + + +.

It is believed that the cross reactions between brain, testis and corpus luteum are due to the presence in their alcoholic extracts of a common antigen whose chemical nature is as yet unknown. Cholesterol, a lipoid found in most tissues, does not seem to be the active element since its concentration in the alcoholic extracts of organs used in these experiments is not consistent with their

antigenic relationships. Cholesterol in each organ extract was determined colorimetrically by the Liebermann-Burchard reaction and expressed in percentages of the solid matter in the extracts. The figures, given in Table III, show that the brain extract contains approximately five times as much cholesterol as do the extracts of testis and corpus luteum, yet brain, testis and corpus luteum are antigenically equivalent as far as the reactions of their lipoids with antiserums for brain, testis and corpus luteum are concerned. The extract of ovary, which gave a feeble reaction at best with these antiserums, contains more cholesterol than the corpus luteum extract. The kidney extract contains more cholesterol than either testis or corpus luteum extract but gives entirely negative reactions with the antiserums tested. Moreover, the extracts of brain, testis and corpus luteum from which cholesterol is removed by precipitation with digitonin retain unaltered their antigenic relationship.

The fact that corpus luteum and testis are sex glands might indicate that sex hormones or their derivatives are concerned with their own antigenic relationship, but would not explain their relationship to brain unless this organ could also be shown to contain sex hormones or chemically similar substances.

TABLE III

Cholesterol Contained in Alcoholic Extracts of Beef Organs

Alcoholic extract of	Amount of solids per 100 cc.	Percentage of cholesterol in the solid fraction
Brain	1.211	25.19
Corpus luteum	0.926	5.22
Testis	0.543	4.35
Ovary	0.714	8.98
Liver	1.557	3.31
Kidney	1.082	7.21
Heart	0.746	3.12
Lungs	0.224	2.89
Spleen	1.444	5.68

CONCLUSIONS

The alcoholic extract of beef corpus luteum gives complement-fixation reactions with antiserums for beef corpus luteum, beef testis and beef brain.

The alcoholic extract of beef ovaries from which corpora lutea have been removed as completely as possible gives negligible reactions with the above antiserums.

The antiserum for beef corpus luteum, in addition to reacting with alcoholic extracts of beef corpus luteum, will also react with extracts of beef brain and beef testis but not with those of liver, kidney, heart, lung or spleen.

An anti-human brain serum will react with an alcoholic extract of corpus luteum and an anti-beef corpus luteum serum will react with an alcoholic extract of sheep brain.

The cross reactions between the alcoholic extracts of brain, testis and corpus luteum are apparently not accounted for by the cholesterol present in the extracts.

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THE INTRINSIC NERVES OF THE IMMATURE HUMAN UTERUS*

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Certain phases of the intrinsic nervous tissue structures of the uterus have been investigated, but many of the finer details have not been explored or have been described without adequate confirmation.

LITERATURE

Remak [1] in 1840, according to Davis,[2] was the first to demonstrate histologically the presence of nerve fibers in the uterine tissues. He described myelinated nerves in the uterus of pregnant animals and unmyelinated nerves in nonpregnant animals. He did not observe nerve cells in the uterus. Many reports cite Lee [3] (1841) as the first to demonstrate nerves in the human uterus. For several years there was an acrimonious correspondence between Lee [4] and Beck [5] over the accuracy of the former's observation.

Kilian [6, 7] in 1851 described myelinated nerve fibers in the uterus. Their distribution was uniform except in the cervix, where the supply was more abundant. He traced the nerves to the mucosal lining. Frankenhäuser [8] in 1867 reported myelinated and unmyelinated nerve fibers in the uterus, the former supplying the blood vessels, the latter passing to the smooth muscle and sending small branches into the cells of the mucosal epithelial lining. Patenko [9] in 1880 described a fine plexus of unmyelinated nerves in the submucosa from which fine fibrils extended to the epithelium. He also reported nerve cells along the nerve fibers in the muscularis. Köstlin [10] (1894) observed multipolar cells in the uterus, similar to ganglion cells, but not connected with nerve trunks. He described a fine nerve plexus in the mucosa of the uterus with delicate filaments extending into the epithelium and ending in small nodes in the individual cells. Clivio [11] in 1894 reported intramuscular and submucous plexuses

731

composed of many fine nerve fibrils and nerve endings in the
epithelium. He noted multipolar cells similar to those described
by Köstlin, but did not regard them as ganglion cells because
they lacked connection with nerve fibers. v. Gawronsky[12]
(1894), in Golgi preparations, interpreted cells in the uterine
wall as nerve structures. He also described bundles of nerves in
the myometrium, which divided into fascicles under the endome-
trium and passed parallel to its surface. Fine branches from
these fascicles ended in nodes in the endometrium. He stressed
the presence of a rich nerve supply of the blood vessels.

Herlitzka[13] in 1897 described three varieties of nerves in the
uterine wall: (1) unmyelinated fibers to the blood vessels, (2)
cerebrospinal nerves with Ranvier's nodes ending intracellularly
and (3) myelinated fibers extending to the smooth muscle. Each
system seemed independent. He described multipolar cells re-
sembling ganglion cells in the uterus, entirely unconnected with
the uterine nerves. Labhardt[14] (1906) found no nerve structures
in the endometrium, but noted nerve fibrils ending in muscle
sheaths, insufficient to supply each muscle cell. La Torre,[15] using
the Cajal method, concluded that the intra-uterine nerves were
unmyelinated. Hoogkamer[16] in 1913 described nerve endings
in the endometrium and fine fibrils extending between the epi-
thelial cells. Though unable to demonstrate intracellular nerve
endings, he found a variety of nerve cells in the uterus which
suggested the presence of an extensive ganglion cell apparatus
in the myometrium and mucosa. Dahl[17] in 1916 demonstrated
myelinated and unmyelinated nerves among the muscle fibers
and about the blood vessels. The external os, according to his
statements, receives only fine unmyelinated fibers, and the cervi-
cal muscle has a rich supply of both varieties of nerves. Dahl
described cone-shaped enlargements in the termination of the
nerves at the muscle fibers. He observed nerve bundles in the
mucosa but no connections with the epithelium, nor could he
demonstrate nerve cells in the uterine tissues. Oudendal[18] in
1922 described small ganglion cells in the uterine wall. Mabuchi[19]
(1924) observed small spindle-shaped nerve endings in the myo-
metrium, but no nerve elements in the endometrium. He con-
cluded that stimulation passes directly from one muscle cell to
another. Gemmell,[20] Fleming[21, 22] and Naiditsch[23] did not find

nerve cells in the uterus. Fleming concluded that the control of
uterine movements is maintained by the intramuscular neurons.

Davis [2] in 1933, using the intravital and supravital methylene
blue method, or modifications of the methods of Cajal, Levaditi,
Bielschowsky, and Prince, demonstrated myelinated and unmyeli-
nated nerve fibers in the uterine tissues arising from nerve bundles
of considerable size at the periphery. In the body of the uterus
the nerve fibers were demonstrated among and parallel to the
smooth muscle fibers and about the capillary blood vessels. Fine
unmyelinated nerves were found in the cervix beneath the epi-
thelium of the cervical canal and of the vaginal portion of the
cervix. Davis was unable to find nerve fibers beneath the mucosa
of the corpus uteri nor endings within the epithelium of any part
of the organ. He emphasized the preponderance of nerves around
the capillaries but failed to observe nerve endings in the vascular
endothelium. He observed the subepithelial nerves below the
vaginal portion of the cervix and considered the complicated
plexus containing many spidery ganglion cells to be associated
intimately with sensation and probably a part of the cerebro-
spinal system. Davis found no ganglion cells regularly in the
substance of the uterus except in this portion of the cervix. He
regarded the microganglia alongside the cervix as sufficient to
perform the so-called automatic action in the separated uterus.

Keiffer [24-26] in 1934–35, using the method of Bielschowsky,
modified by Reumont and himself, described macroganglia and
microganglia in the retrosphincteric connective tissue of the lower
uterine segment. This included the sphincter with the mucosa
of the internal orifice and all of the connective tissue surrounding
this sphincter for a height of about 5 mm. and generally extending
to the vaginal cul-de-sac. The ganglion cells apparently corre-
spond to those described by others in the so-called cervical gan-
glion. Isolated nerve cells within the uterine wall appeared to
be unipolar. Keiffer described several structures in the retro-
sphincteric connective tissue and within the uterine wall which
he considered to be sensory corpuscles or nerve receptors. They
appeared to be specially adapted to the physiology of the muscles
because of their location between interwoven muscle bands.
Others, associated with blood vessels, were regarded as chemical
receptors.

MATERIAL AND METHODS

The preceding review demonstrates the limited accurate information concerning the intrinsic nervous tissue structures of the uterus and the divergence of opinions concerning the anatomic details. Apparently one of the main obstacles has been the inadequacy of the staining methods used, none being sufficiently differential for the nerve elements.

A study of the nervous tissues of the uterus was initiated by applying to immature uteri the methods used in other studies of the peripheral nervous system by Masson [27] and Popoff.[28] Uteri from four infants constituted the material: one was from a fetus about 3 months premature; the second from an infant 4 months of age; the third from a child 2 years of age; the fourth from a child of 9 years. They were removed with contiguous structures such as broad ligaments, fallopian tubes, vagina and regional connective tissues, and fixed in Bouin's solution. Each uterus was embedded in paraffin and sectioned serially at 6 to 10 μ. Four to eight sections were mounted on each slide. As every second or third slide was stained, depending upon the number of sections on a slide, the gaps of unstained sections did not exceed 80 μ. All sections of the uterus from the child 9 years of age were stained. Goldner's [29] modification of Masson's trichrome stain was used. Sections of a gasserian ganglion of an adult were used as controls in the staining procedures. The axis cylinders of the nerve fibers stained red. Myelin, if present, was dissolved to leave a clear space about the axis cylinder. The supporting protoplasmic network in this space stained a faint purple-red. All collagenous fibers stained light green. The fibrous connective tissue nuclei stained dark blue, the red blood cells orange-red and the smooth muscle fibers of the uterus brick-red.

OBSERVATIONS

The general distribution of the nerves was similar in all uteri examined. The large nerves approached the uterus on both sides lateral to the cervix at and slightly above the level of the vaginal cul-de-sac. They were accompanied by many large blood vessels and were surrounded by loose fibrous connective tissue. At various levels corresponding to the upper part of the cervical canal and lateral to the internal os, these large nerve trunks had fusi-

form dilatations with many small and medium-sized ganglion cells. Most of the nuclei were ovoid or slightly irregular, without a clearly demonstrated nucleolus. Many of the large nerve trunks entered the wall of the cervix with large blood vessels. The larger nerve trunks seemed to parallel the cavum of the uterus. Smaller branches from these trunks extended at angles toward the cavum of the cervix. In the cellular submucosa supporting the epithelial lining of the cervix these nerves branched into numerous small interlacing twigs usually composed of only one axis cylinder just beneath the columnar lining. Fibers did not extend into the epithelial cells. This fine nerve network apparently corresponds to the subepithelial plexus of the cervix described by Davis.[2]

Most of the large nerve trunks, deep within the wall of the cervix, extended along the long axis of the uterus into the body portion. Others, accompanied by large blood vessels, passed along the lateral side of the uterus in the tissues corresponding to the attachment of the broad ligament to higher levels of the corpus of the uterus. Some of these gradually passed through the outer layers of the myometrium to the anterior or posterior surface of the body of the uterus, then into the deeper portions of the myometrium. The number of large nerve trunks decreased progressively toward the fundus. The lamina propria or submucosa of the body of the uterus in the material studied did not have the extensively branching network of fine nerve fibers present in the submucosa of the cervix. Many small nerve fibers could be traced into the submucosa but not into the epithelial lining cells.

In all uteri examined, close association of the nerves and blood vessels existed. This was indicated by the proximity of the large nerves and blood vessels as they entered the uterine wall, often in the same connective tissue bundle. The relationship was maintained occasionally even in the smaller capillaries and nerve fibrils. In these small uteri the endings of axis cylinders in the walls of the blood vessels could not be demonstrated. In some places fibrils seemed to branch from a nearby nerve and to encircle small blood vessels.

Although numerous ganglion cells were present in the region of the so-called cervical ganglia on the lateral sides of the cervix,

none was within the uterine wall. A few cells in the course of some of the smaller nerve trunks in the body portion of the uterus resembled in structure and staining qualities those in the cervical ganglia. Other similar cells, perhaps ganglion cells, were at the bifurcation of small nerve trunks in the body portion of the uterus. These nerve trunks at the levels of bifurcation became more cellular. In the open part of the Y-shaped structure formed, there were several oval, red-purple nuclei with considerable chromatin and a small nucleolus surrounded by an irregular homogeneous purple cytoplasm. The appearance and staining qualities of these cells resembled nerve cells in the cervical ganglion but, not being found regularly, their interpretation as ganglion cells is doubtful.

COMMENT

Some problems confronting an investigation of the intrinsic nerves of the human uterus should be mentioned briefly. The first is of a technical nature and concerns tissue stains and the material used. Many of the staining methods, such as the silver and methylene blue methods, although excellent for demonstrating cellular detail, do not differentiate the various tissues, nor are they conveniently adaptable to serial sections. Goldner's modification of Masson's trichrome stain may be used with serial sections and gives excellent differentiation of the tissues, despite some loss of the finer cellular details. In the uterine tissues examined, the brick-red color of the smooth muscle fibers contrasted with the light green of the fibrous tissue. The smooth muscle cells in the walls of the blood vessels were recognized easily even in tangential sections. They were not confused with nerves longitudinally cut which have wavy green lines and slender purple sheath cells. Difficulties in distinguishing nerves from blood vessels occur with the smallest capillaries and single nerve fibrils. A single red blood cell within the lumen of such a capillary occasionally may be orange-red as is a single axis cylinder. Other difficulties in the interpretation of these structures usually can be avoided by tracing them in serial sections. The infantile uterus is suitable especially for serial sections. The principal disadvantages in its use are the immature development, or the slight alteration of staining qualities of the nervous tissue and

the failure to demonstrate the axis cylinders with the same precision as in the gasserian ganglion of an adult.

The presence of nerve cells or other structures which may initiate or maintain the so-called automatic action and tone of the uterine musculature is not established. Davis [2] believed that sufficient microganglia are distributed along the cervix to provide the mechanism necessary for the so-called automatic action in the isolated uterus. Keiffer [24-26] considered the nerve cells in the retrosphincteric connective tissue in the lower segment, apparently the cervical ganglia, the reflex center for the uterus. The experiments of Fleming [21, 22] and Kaminester and Reynolds,[30] demonstrating that the tone and movements of the uterus are independent of the cervix and cervical ganglia, provide evidence that the origin of the controlling nervous impulses is above the level of the cervix and cervical ganglion. Some authors have described nerve cells within the wall of the uterus from which such impulses could arise. Others have failed to corroborate this observation.

SUMMARY

The distribution of the nerves in the infantile uteri examined resembles that described by other investigators. Nerves pass from the cervical ganglia on the lateral sides of the cervix into the wall of the uterus accompanied by large blood vessels. The larger nerve trunks extend parallel to the long axis of the uterus and lie deep within the myometrium. Small branches extend from these trunks to the endometrium, without entering the epithelial lining cells. They form an intricate network or plexus in the lamina propria of the cervical canal. The lamina propria of the body of the uterus has fewer nerves than the cervix. The nerve trunks to the upper portion of the body of the uterus ascend in the connective tissue of the broad ligaments or in the superficial layers of the myometrium at the periphery of the uterine wall. The number of nerve trunks diminishes progressively toward the fundus of the uterus. The significance of the close anatomic association of the nerves and blood vessels within the uterine wall is not clear. A similar relationship exists between the nerves and blood vessels in other tissues of the body. Nerve endings in the vascular walls were not demonstrated. No tissues

were found which could be interpreted as sensory corpuscles within the uterine wall or nearby connective tissues.

NOTE: Since the completion of this report a study of nerves in the adult human endometrium has been accepted by the *Archives of Pathology* for publication. The nerve trunks in the myometrium beneath the endometrium contained mainly unmyelinated fibers and also one or several myelinated fibers.

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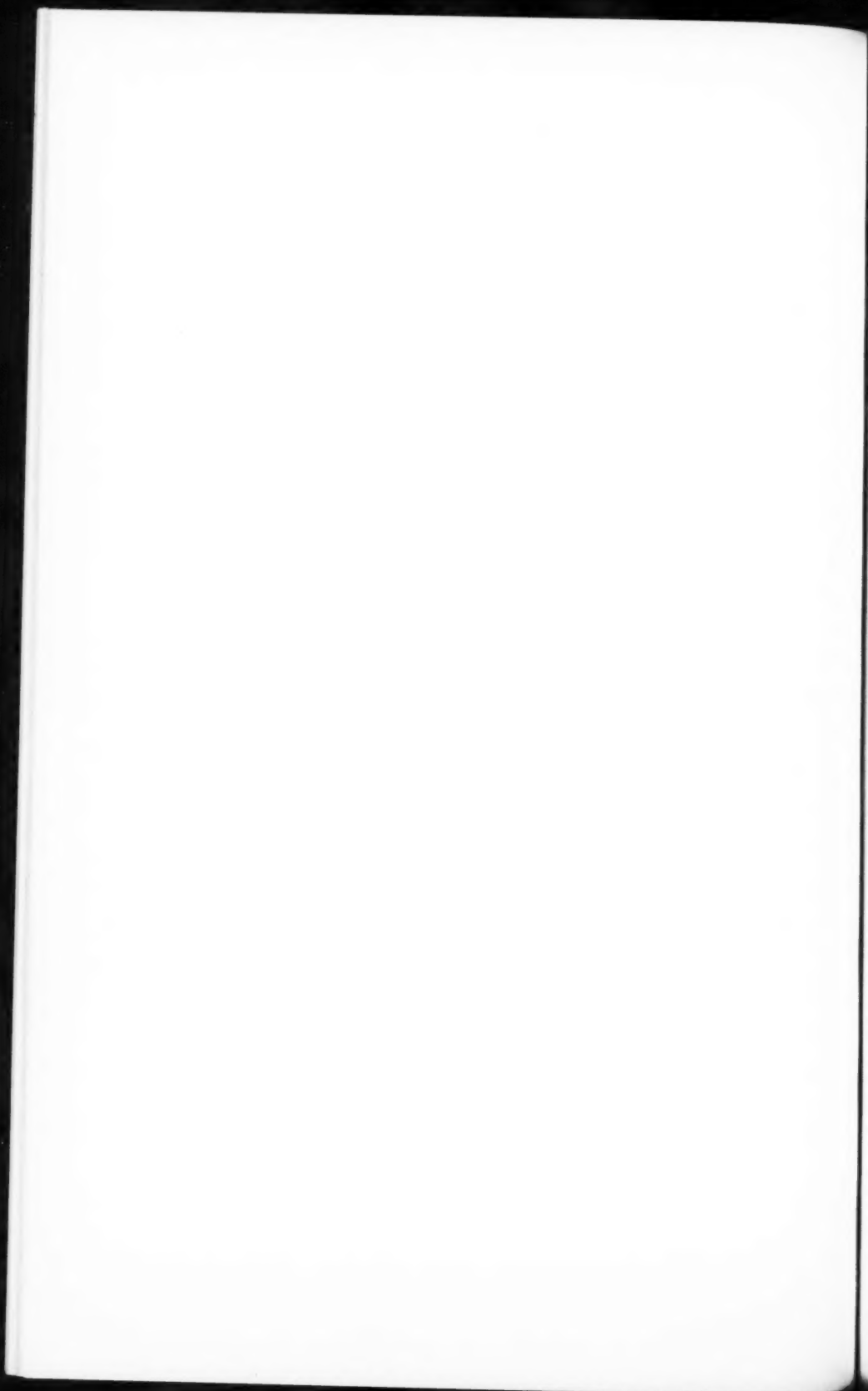
SOME FACTORS IN THE DEVELOPMENT, LOCALIZATION AND REABSORPTION OF EXPERIMENTAL AMYLOIDOSIS IN THE RABBIT*

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Certain aspects of the problem of amyloidosis have engaged our attention for a number of years. This paper is a summary of our findings, and is dedicated to a great teacher and investigator of pathology whose manifold interests also include experimental amyloidosis.

METHODS

Healthy rabbits, weighing between 1500 and 2000 gm. and kept on an adequate mixed diet, were injected intravenously with strains of hemolytic streptococci freshly isolated from the upper respiratory passages of patients with acute glomerulonephritis. Scarlet fever streptococci, green-producing streptococci from the respiratory passages or urines of patients with chronic nephritis and systemic lupus erythematosus, pneumococci of types I and III, and a Friedländer bacillus were also used, along with some other strains, including a hemolytic streptococcus of canine tonsillar and endocardial origin. All organisms were grown in blood broth.

A series of rabbits was also injected with scarlet fever antitoxin, horse serum, rabbit serum, rabbit plasma albumin and rabbit plasma globulin.

Material for histologic study was fixed in a 10 per cent aqueous solution of formaldehyde and in Zenker's solution and stained with Congo red for amyloid and with hematoxylin and eosin for general purposes. Ordinarily, kidney, liver, spleen, adrenal and myocardium were sectioned; occasionally, other organs.

Determinations of plasma proteins, blood urea and nonprotein nitrogen, and plasma cholesterol were made by the usual clinical microchemical methods. Albuminuria was estimated with the heat and acetic acid test and at times by quantitative chemical analysis.

RESULTS

Amyloid was found in the kidneys, liver or spleen (or in several of these organs) in 104 of the 181 rabbits injected with bacteria, or 57 per cent. In general, rabbits injected with strains of virulent hemolytic streptococci, Friedländer's bacilli or pneumococci developed amyloid sooner and more extensively than animals given the relatively avirulent green-producing streptococci or other organisms long after their isolation from human cases. In the case of staphylococci, septicopyemia killed the animals in too short a period to permit development of amyloidosis. The dosage used may have been too large, for other investigators [1-3] have produced amyloidosis in rabbits with this organism. No other organism employed failed to bring about the deposition of amyloid in some rabbits.

The histology of experimental amyloidosis has been repeatedly described and we have alluded to it in earlier preliminary reports.[4, 5] In a large majority of rabbits coming to autopsy before amyloidosis has had time to develop, marked lymphoid and reticular cell hyperplasia of the splenic follicles has been observed, often with numerous mitotic figures. The amyloid was always extracellular.

Factors in the Distribution of Amyloid

The localization of amyloid was characteristic. In the kidney, amyloid was found only in the glomerular tuft and along the medullary capillaries. In the spleen, amyloid was usually deposited in the periphery of the follicle, extending inward and outward as it increased. The follicular arterioles were only slightly involved. In the liver, amyloid was observed about the peripheral sinusoids, extending centrally and producing the typical atrophy. The adrenal gland exhibited amyloid around capillaries in the zona reticularis near the medulla, spreading later toward the zona fasciculata. In no case was the entire adrenal cortex infiltrated nor extensive atrophy of tissue produced. Amyloid was never found in the myocardium, lungs, thymus or aorta. It was sometimes seen in the tail of the pancreas, involving the capillaries of both the ordinary and islet parenchyma. A few observations of the stomach and intestines showed amyloid about the capillaries of the mucosa.

The relative distribution of amyloid in the main sites of occurrence—kidney, spleen, liver and adrenal—was determined largely. by the total length of the experimental period and the duration of survival after the injection period (Table I). Thus, grading the amount of amyloidosis on a basis of 4 plus as a maximum in each organ and classifying the rabbits into groups, the following distribution was obtained: (a) No amyloid in 80 rabbits living less than 7 weeks. There were a few animals without amyloid living longer periods, up to 38 months. (b) Moderate to considerable amyloid in the spleen (or liver) with little or none in the kidneys in 24 rabbits, of which 9 died in 4 to 8 weeks, 12 in 3 to 5 months, none in 6 to 11 months and 3 in 12 to 18 months. (c) Moderate to considerable amyloid in the spleen, kidneys and often the liver, less often in the adrenal, in 26 rabbits, of which none died prior to 8 weeks, 13 in 2½ to 6 months, 4 in 7 to 11 months and 9 in 12 to 24 months. (d) Moderate to considerable amyloid in the kidneys and often the adrenals, with little or none in the spleen or liver in 36 animals, of which 1 died in 2½ months, 1 in 5 months, 14 in 7 to 11 months and 20 in 12 to 30 months.

TABLE I

Distribution of Amyloid According to Duration of the Experimental Period

Period	Total No. of rabbits used	No. without amyloid	With generalized amyloidosis	With amyloid chiefly splenic, little or none in kidneys	With amyloid chiefly renal, little or none in spleen
1–2 mo.	77	68	0	9	0
3–6 mo.	31	4	13	12	2
7–11 mo.	20	2	4	0	14
12–30 mo.	35	3*	9†	3‡	20‖

Eighteen rabbits used in the experiment are not included in this table since, while they showed slight amounts of amyloid, there was too little to consider organ-distribution significant.
* 12–38 months.
† 12–24 months.
‡ 12–18 months.
‖ 12–30 months.

Apparently, bacterial amyloidosis develops first in the spleen and liver and later in the kidneys with subsequent diminution or disappearance of splenic and hepatic amyloid but an increase in renal and, probably, in adrenal amyloid.

In 10 rabbits bacteria were injected in two separate courses, 8 to 10 months apart in 6 animals and from 1½ to 3½ months apart in 4 animals. In 2 out of the 3 rabbits in the group (b)

showing predominance of splenic amyloid in spite of a total
experimental period of 12 to 14 months, the time elapsed from
the beginning of the second injection course to death was 4¾
and 2 months, respectively, or long enough to permit the deposi-
tion of amyloid. In the 4 rabbits in group (c), 3 had total
experimental periods of 10 to 15 months, with the second experi-
mental periods of 1, 4½ and 5 months, respectively. In this
group either the first or the second injection courses could have
produced the amyloid in 2 of the 3 rabbits. On the other hand,
3 rabbits in group (d) had total experimental periods of 5, 17
and 28 months, respectively. In the latter 2 rabbits there was
no amyloid in the spleen, but the second experimental periods
lasted 10 and 16 months, respectively, or long enough to explain
the predominantly renal amyloid.

Factors in the Development of Amyloidosis

The duration of bacterial injections seems to have little relation
to the development of amyloidosis except for the longer period
required in the case of avirulent strains. In our earlier experi-
ments,[4] it was found that freshly isolated and virulent strains
of hemolytic streptococci from cases of acute nephritis produced
albuminuria (renal amyloid) much sooner than did the less
virulent or older strains. Without frequent biopsies on the spleen
and in the absence of albuminuria it is impossible to determine
the exact onset of amyloidosis. However, in 17 rabbits with well
developed amyloidosis the entire injection period extended over
3 weeks or less, in 6 animals for only 3 to 4 days. Yet in this
group the total experimental period was 1½ to 3 months in
4 out of 5 rabbits injected with freshly isolated strains of "ne-
phritic" hemolytic streptococci, and from 12 to 26 months in all
9 rabbits injected with freshly isolated strains of *Streptococcus
viridans* or other avirulent organisms.

The rôle of chronic infection and suppuration was not clearly
significant in our series of positive animals. Disregarding termi-
nal infections in the lung, only a small percentage of our rabbits
had vegetative endocarditis, suppurative arthritis, cholecystitis,
pericholecystitis or hepatitis, or chronic pneumonitis. The rabbits
without amyloid were infected to about the same extent as the
positive animals. The etiologic significance of small infarcts in

the kidney seemed dubious. Rabbits with suppuration due to staphylococci died too soon to develop amyloidosis.

Reabsorption of Amyloid

Apart from direct biopsy evidence available in a small series of rabbits,[5] strong indirect support of the reabsorption theory is given by the data on the distribution of amyloid in the kidneys, spleen, liver and adrenals in relation to the total experimental period (Table I). In the spleen, the process of reabsorption seems to be largely the result of invasion of leukocytes, polyblasts and capillaries from the adjacent pulp. The amyloid loses its staining power, is broken up into fragments and gradually disappears. Participation of foreign body giant cells in this process [2, 6-8] was limited to 3 animals although a few more showed giant cells, presumably megakaryocytes, unrelated to the amyloid masses. Following the reabsorption of splenic amyloid the follicles remained atrophic and at times definitely fibrotic. The liver showed little or no residual portal fibrosis. No clear instance of absorption of amyloid in the adrenal has been found in our series.

Evidence for reabsorption of renal amyloid in the rabbit has not appeared in our extensive study. Once the glomeruli and medullary capillaries have become moderately involved, tubular dilatation and degenerative changes set in with later atrophy and ultimate fibrosis. The kidneys usually remain large in spite of fibrosis because of the persistent amyloid, and weights two or three times the normal are a regular occurrence even in the late stages of renal disorganization. The rôle of tubular obstruction in parenchymal atrophy is important.

Experimental Hyperglobulinemia and Amyloidosis

This aspect of the problem has been studied in several ways. First of all, data have been obtained on the variations in the plasma proteins during and after the injection of bacteria in rabbits with or without amyloid. The average control plasma albumin in 73 rabbits was 4.28 ± 0.40 gm. per cent, with 11 values below 3.88 and 17 above 4.68 gm. per cent. The mean control plasma globulin in this series was 1.70 ± 0.41 gm. per cent, with 14 values below 1.29 gm. per cent and 11 values above 2.11 gm. per

cent, 5 being over 2.93 gm. per cent. These globulin figures are slightly lower than those we [5] reported on another series of 46 rabbits with an average control level of 1.86 ± 0.37 gm. per cent.

During the period of bacterial injection, if not less than 3 weeks, the plasma globulin rose to 3 gm. per cent or higher in 34 out of 40 rabbits studied. Elevated plasma globulin was maintained during the longer injection courses.

The plasma globulin values after the period of bacterial injection varied considerably in different rabbits and often in the same rabbit. In 15 animals, the plasma globulin exceeded 2.5 gm. per cent during 2 months or longer, often reaching levels of 3.5 to 4 gm. per cent. However, in 14 rabbits the plasma globulin remained for several months below 2.5 gm., often below 2.0 gm. per cent. No clear difference in the degree or distribution of amyloidosis was found in these two groups, but 5 out of 8 rabbits without amyloid had low plasma globulins. Finally, a number of animals showed irregular fluctuations over a period of months. In the rabbits receiving two courses of bacteria, the second injection period usually was followed by a sharper rise in plasma globulin than the first. Albuminuria, sufficient to lower the plasma albumin to 2.5 gm. per cent or less, was usually associated with an elevation of plasma globulin to 2.5 gm. per cent or more, apart from the possible influence of bacterial injections. The total plasma protein usually reached 7 to 8 gm. per cent when the globulin fraction increased, except in cases of low plasma albumin secondary to albuminuria, when the plasma proteins fell to 5 or 4 gm. per cent.

The plasma globulin was also elevated by the repeated intravenous injection of scarlet fever antitoxin (horse). Of 5 rabbits injected over a maximum period of 1½ months, 3 died of pulmonary edema, 1 lived 10 months and 1 for 2 years (after a single injection). None showed amyloid but all had marked hyperplasia of the spleen. The plasma globulin values in the 4 rabbits studied reached 3.36 to 5.94 gm. per cent during the period of injection, the rise beginning within a week. In the 2 rabbits that survived the injection period, the plasma globulin figures remained within normal levels.

Direct elevation of the rabbit's plasma globulin was made by intravenous injection of rabbit whole serum and rabbit plasma

globulin prepared in various ways. Five rabbits were given serum for periods up to 7 months. Three animals with a total of 1160 to 3000 cc. of serum during 5 to 7 months, with total experimental periods of 9, 16 and 19 months, showed slight to moderate amyloidosis in the kidneys at autopsy, although no amyloid was detected in either the kidney or portion of spleen removed at 5, 1½ and 5 months, respectively. Hyperplasia of the splenic follicles was found in the biopsy specimens. The plasma globulin in the 4 rabbits studied was maintained at 3 to 4 gm. per cent during the period of injection except when the amount of serum was reduced. The plasma albumin also rose so that the total protein reached 8 to 9 gm. per cent. When the serum was discontinued, the plasma globulin promptly fell to normal.

Rabbit plasma globulin was injected intravenously for periods of 1½ to 4 months in 6 rabbits. Considerable splenic or renal amyloid developed in 4 animals, a trace in 1 and none in 1. The definitely positive results were obtained with the more denatured globulin preparations. The plasma globulin rose during the injection period to 3 to 4 gm. per cent in all the rabbits, and persisted at a high level as long as globulin was given in adequate amounts. The highest plasma globulins, 4.6 to 5.9 gm. per cent, for periods of 1½ and 2½ months, were observed in the 2 animals with no amyloid. The plasma globulin fell to normal within a few weeks after the injection period except in the rabbits with persistent albuminuria due to renal amyloidosis. In 2 rabbits given the heat-denatured globulin many giant cells containing amyloid were seen in the spleen.

Dietary Experiments

A few experiments involving the injection of bacteria into rabbits kept on diets free from ascorbic acid or consisting of hay alone revealed no definite influence upon the production or course of amyloidosis. However, in 1 rabbit kept as a control on a hay diet for 10 months and surviving another 7 months on the regular diet, considerable amyloid was present in the kidneys without an obvious focus of inflammation. No amyloid has been observed in any control rabbit on the regular diet up to 3 years nor in rabbits used as blood donors.

Proteinuria

Albuminuria, if persistent, practically always indicated renal amyloidosis.[4, 5] In general, the degree and duration of proteinuria were proportional to the amount of glomerular amyloid. No albuminuria was found in rabbits with considerable amyloid in the spleen or liver but without renal amyloid. However, the absence of proteinuria did not exclude the presence of traces or small amounts of amyloid in the kidney, as was demonstrated in 7 rabbits. Following proteinuria, the plasma albumin fell to 2 gm. per cent or less, the plasma globulin often rose to 2.5 gm. per cent or more, the weight went down and the plasma cholesterol increased temporarily. In several animals the hypo-albuminemia caused transudation into the serous cavities.

COMMENT

Our experiments on some 200 rabbits have demonstrated the facility of production of amyloidosis in this animal by means of intravenous injection of bacteria. This is in agreement with the scattered results of previous investigators.[1, 2, 10-12] The most rapid and most extensive amyloidosis has followed the use of hemolytic streptococci freshly isolated from inflamed upper respiratory passages and tonsils of individuals with acute, recurrent subacute, or active chronic glomerulonephritis. Other organisms have also proved effective in initiating amyloidosis when freshly isolated. After ageing on laboratory media, larger doses and more prolonged injections are necessary. In a great majority of our positive experiments, no focus of chronic suppuration or inflammation was found to account for the apparently progressive evolution of amyloidosis long after injections of bacteria had ceased and long after they must have disappeared from the animal body. In several rabbits, only three or four daily injections of bacteria sufficed to produce amyloidosis.

A constant and striking feature in this study was the change in distribution of amyloid with the lapse of experimental time. Splenic amyloid predominated markedly over renal amyloid in practically all of the rabbits coming to autopsy within 2 months of the onset of the experiments. Animals with periods of 2 to 6 months were very likely to show more or less uniform amyloidosis in the spleen, kidneys and liver. After 7 to 11 months the

tendency increased for marked renal and adrenal amyloid with little or none in the spleen and liver. The apparent exceptions of behavior could be reasonably explained as the deposition of amyloid in response to a second, or more recent, course of injection of bacteria. In several instances biopsies of the spleen and kidney gave direct confirmation of the later change from predominantly splenic to predominantly renal amyloid.

These observations on the reabsorption of amyloid in the spleen and liver confirmed previous reports on reversibility of amyloidosis in these organs in the mouse,[8] rabbit,[2,3] horse [7] and man.[2,6,13,14] However, in spite of discontinuance of bacterial injections and the disappearance of splenic amyloid, renal amyloid not only remained but actually increased, ultimately causing disorganization of parenchyma and varying degrees of fibrosis and functional impairment. While the situation may be different in human renal amyloidosis, the literature is not convincing.[6,13,14] Physiologically, conditions are more favorable for the reabsorption of amyloid in the spleen and liver than in the kidney.

There has been much speculation concerning the chemical nature of amyloid, the mechanism of its precipitation in the walls of arterioles and capillaries, and the relation of the processes of immunity, including hyperglobulinemia, to the pathogenesis of amyloidosis. A recent excellent study of the physical chemistry of human amyloid [15] illustrates the difficulties involved. The rôle of chondroitin-sulfuric acid is still an intriguing problem.[16] Such factors as hyperglobulinemia, the circulating or local precursors of amyloid, antigen-antibody precipitation and others have been discussed at some length by previous investigators.[17-21] It is generally agreed that any type of prolonged cellular stimulation by foreign protein of external or internal origin leads to both hyperglobulinemia and amyloidosis in a highly susceptible animal, like the mouse or the rabbit, or the horse used for the production of immune sera.[7] The apparent rarity of amyloidosis in individuals with kala-azar or lymphogranuloma inguinale, in which very high plasma globulin levels often occur, is difficult to understand.

Our direct attempt to test the theory of hyperglobulinemia as a cause of amyloidosis by injecting whole rabbit serum or concentrated rabbit plasma globulin in 10 rabbits yielded conflicting

results difficult to interpret, unless we assumed that the method of preparation of the serum proteins was such as to denature them for the rabbit. In that event the absence of amyloid in the 2 animals given the best globulin preparation is strong evidence against the simple assumption of hyperglobulinemia as a cause of amyloidosis. It has been reported,[18] without details, that electro-ultrafiltered rabbit globulin produced neither amyloid nor precipitins in rabbits. More experiments are necessary, including identification of the plasma globulin during the formation of amyloid.

The more indirect attempts to correlate hyperglobulinemia and amyloidosis [5, 17, 21] cannot, in our experience, lead to a conclusive decision. Whether amyloid is formed or not, the parenteral injection of antigen stimulates a rise in plasma globulin, relative or absolute. The plasma globulin level may be markedly increased or normal for months after the injection period in rabbits with or without amyloidosis. The low figures cannot be explained, as has been suggested,[21] on the basis of proteinuria or hepatic amyloidosis. A second course of injections of bacteria or other antigen usually leads to a rapid rise in the plasma globulin level even in rabbits which failed to respond to the first series.

Some miscellaneous observations are of interest. The incidence of gross aortic medial necrosis, calcification and atheromatosis [9] in the amyloid rabbits was 12 per cent, six times as high as in the nonamyloid and control groups. Anemia and loss of weight were frequent concomitants of the process of amyloidosis and were intensified during periods of considerable proteinuria. Anemia itself did not cause amyloidosis in a series of blood-donor rabbits. The spleen often contained many erythrocyte-laden and blood-pigment-laden macrophages. In rabbits with experimental periods of a year or longer, renal amyloidosis was usually associated with marked tubular atrophy and obstruction, fibrosis of the parenchyma and hyalinization of the glomeruli containing amyloid. In spite of this "contraction" of the kidneys, the weight still exceeded the normal because of the amyloid content. Amyloid was practically never found in any renal vessels other than the intraglomerular arterioles.

SUMMARY

1. Amyloidosis has been produced in a large series of rabbits by the injection of various bacteria from human sources.

2. Splenic and hepatic amyloid appear early, but can also disappear in time. Evidence of active reabsorption of amyloid is presented.

3. Renal amyloid develops later but tends to increase with time to the point of extreme disorganization and fibrosis of the parenchyma, and functional insufficiency. There is no evidence of absorption of renal amyloid in the rabbit.

4. Hyperglobulinemia, relative or absolute, is a constant finding during the longer periods of bacterial injection, but may or may not persist in the after period. Albuminuria may elevate the plasma globulin relatively.

5. Artificial hyperglobulinemia, the result of injections of rabbit serum or globulin, does not regularly produce amyloidosis. The positive results may be secondary to denaturation of the serum proteins. Presumably, other factors than hyperglobulinemia are necessary for the development of amyloidosis.

6. Amyloidosis may appear and progress in the absence of ordinary signs of inflammation or suppuration in the rabbit.

7. Gross aortic disease in the form of medial necrosis and calcification or atheroma is six times as prevalent in rabbits with amyloidosis as in the nonamyloid series. Atheroma is always associated with persistent hypercholesterolemia.

8. The pathogenesis of amyloidosis is not adequately explained by the prevailing theories and requires further investigation.

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DESCRIPTION OF PLATE

PLATE 118

FIG. 1. Moderate amyloidosis in the kidney (biopsy) of rabbit No. 9, injected with a scarlatinal streptococcus for 10 months and showing albuminuria for 3 months. The glomeruli contained amyloid in amounts varying from 1 to 4 plus. The proximal convoluted tubules are atrophic and the distal tubules markedly dilated. There is some increase in interstitial tissue. Hematoxylin and eosin stain. × 170.

FIG. 2. Marked amyloidosis in glomeruli, and degeneration of tubules in rabbit No. 3, injected with a "nephritic" hemolytic streptococcus for 3½ months in two courses, with a total experimental period of 6½ months and persistent albuminuria in the last 1½ months. Congo red stain. × 170.

FIG. 3. Marked glomerular amyloidosis, tubular atrophy and obstruction, and interstitial fibrosis in rabbit No. 11, injected with a type I pneumococcus for 6 months. Albuminuria appeared at 2½ months and persisted during the 5 months after the injection period. Hematoxylin and eosin stain. × 63.

FIG. 4. Marked glomerular amyloidosis and fibrosis of parenchyma in rabbit No. 312; injected with *Streptococcus viridans* for 4 months and surviving another 14 months, with albuminuria during most of this period. The spleen and liver showed only traces of amyloid. Van Gieson's stain. × 75.

FIG. 5. Medial necrosis and calcification, and intimal fibrosis in aorta of rabbit No. 535, injected with a "nephritic" hemolytic streptococcus for 7 weeks in two courses, with a total experimental period of 10 months. There was generalized amyloidosis. The aorta was calcified as far as the origin of the renal arteries. Hematoxylin and eosin stain. × 18.

FIG. 6. Atheromatous plaque in ascending aorta of rabbit No. 360, injected with a Friedländer bacillus during 1½ months and surviving another 14 months. There was marked generalized amyloidosis, with fibrotic kidneys. The plasma cholesterol ranged between 179 and 352 mg. per cent for a year. Van Gieson's stain. × 250.
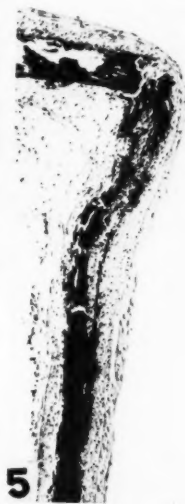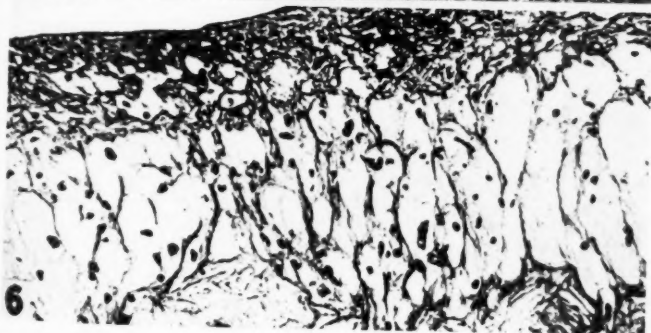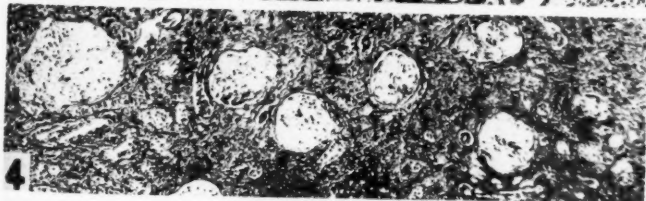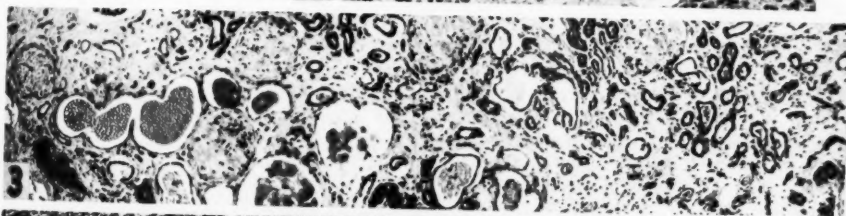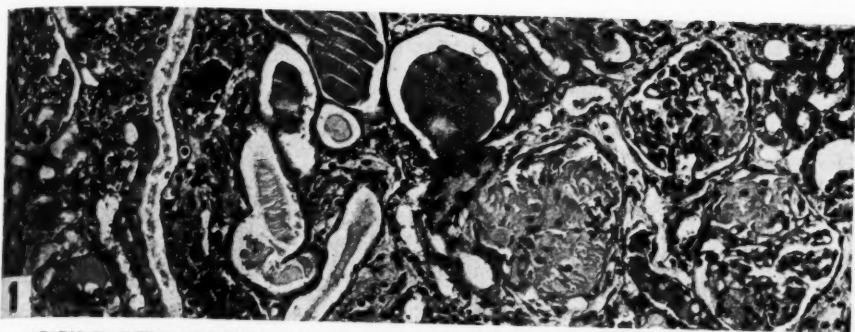
FATTY CHANGES IN THE GLOMERULI OF THE KIDNEYS *

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In the course of experiments designed to produce glomerulo-nephritis in dogs, it was found that the glomeruli in many of these animals contained stainable fat. References in the literature to the presence of fat in the glomeruli consist of widely scattered casual observations usually without exact descriptions and are relatively few in number. We have attempted to review the literature, to analyze the occurrence of this condition in our own material from autopsies and from experimental animals, and to arrive at some understanding of its significance.

No adequate data on the frequency of visible fat in the glomeruli are available. Its presence is probably always pathological although Lubarsch suggested that it may sometimes be physiological. Fahr stated that it is associated with lipemia and that in the glomeruli it is an infiltration rather than a degeneration. Lubarsch mentioned, without giving details, fatty changes in the capsular epithelium in 64, in the glomerular epithelium in 16 and "fine dust-like fat" in 232 of 2,720 autopsies, or slightly less than 11.5 per cent. Prym found fat in the glomeruli in 42 out of 211 autopsies, or 19.9 per cent; and Segawa in 46 out of 150 autopsies, or 30.7 per cent. In 28 of Segawa's cases the amount of fat in the glomeruli was described as moderate; in 14, as abundant; and in 4 as very abundant. It is evident from these observations, as well as our own, that fat in the glomeruli is not uncommon in various pathologic conditions. But even with satisfactory stains the fat may be overlooked because of the extreme fineness of the droplets. Diseases in which fatty changes in the glomeruli have been recorded are tabulated as follows:

Diseases in Which Fat Has Been Found in the Glomeruli

Toxemias and poisonings:
  Acute yellow atrophy of the liver: Mayer (1922).
  Alcoholism: v. Kahlden (1893).
  Bichloride of mercury: Heineke (1909); Bohnenkamp (1922).
  Burns: v. Kahlden (1893).
  Eclampsia: Prym (1910); Segawa (1914); Fahr (1925).
  Phenol poisoning: Peipers (1892).

Metabolic disturbances:
  Beriberi: Segawa (1914).
  Diabetes mellitus: Peipers (1892); Prym (1910); Segawa (1914);
    Fahr (1920); Lubarsch (1925).
  Exophthalmic goiter: Prym (1914).
  "Arteriosclerosis": Prym (1910); Segawa (1914).

Circulatory disturbances:
  Anemia: Segawa (1914).
  Passive hyperemia: Segawa (1914).
  "Heart disease": Prym (1910).

Other diseases:
  Cirrhosis of the liver: Segawa (1914).
  Jaundice: Prym (1910); Segawa (1914).
  Purpura hemorrhagica: Segawa (1914).
  Malignant tumors: Prym (1910); Segawa (1914).
  Amyloidosis: Tietz (1922); Van Slyke (1930).

Infectious diseases:
  Diphtheria: Nauwerck (1886); v. Kahlden (1893); Segawa (1914);
    Fahr (1920).
  Dysentery and enteritis: Prym (1910).
  Erysipelas: Segawa (1914).
  Meningitis: Prym (1910); Herxheimer (1916, 1918).
  Peritonitis: Prym (1910); Segawa (1914).
  Pneumonia: Peipers (1892); Gaskell (1911); Segawa (1914).
  Scarlet fever: Löhlein (1904).
  Sepsis: Prym (1910).
  Tuberculosis: v. Kahlden (1891); Prym (1910); Segawa (1914).
  Typhoid fever: v. Kahlden (1893); Gaskell (1911).

Renal diseases:
  Acute glomerulonephritis: Munk (1918); Stern (1924); Volhard
    (1925); Hückel (1929).
  Chronic glomerulonephritis: Peipers (1892); Löhlein (1905); Prym
    (1910); Segawa (1914); Volhard (1925); Fahr (1925); Van Slyke
    (1930); Gray (1933).
  Nephrosclerosis: Prym (1904); Gaskell (1911); Herxheimer (1912,
    1916, 1918); Jores (1916); Munk (1918); Tietz (1922); Van Slyke
    (1930); Gray (1933); Kimmelstiel and Wilson (1936).
  Nephrosis: Löhlein (1918); Munk (1918); Major and Helwig (1925);
    Fahr (1925); Löwenthal (1927); Bell (1929); Kantrowitz and
    Klemperer (1931).
  "War nephritis": Herxheimer (1916, 1918); Rochs (1918).

Of the authors listed, only Jores (1904, 1916), Prym (1904, 1910), Herxheimer (1912) and Segawa (1914) make more than casual comment upon the presence of fat in the glomeruli. The greater part of the references were found by examining the descriptions of microscopic sections of the kidneys in reported cases of the various diseases.

There is no complete agreement as to the location of fat in the glomeruli. Bell, Jores, Aschoff and Kaufmann state that it is in the epithelium; Rochs, Löwenthal and others, that it is in the endothelial cells. Normally the epithelial cells of the glomeruli greatly outnumber the endothelial cells. But in glomerulonephritis, the latter are increased by proliferation. This renders the identification of cells containing fat more difficult. The only cells in which it can be accurately observed are those which line the capsular space.

We have studied sections of kidneys stained with Sudan III from 76 autopsies and from 133 dogs without attempting to differentiate between neutral fats and lipoids. The autopsies were selected largely because they involved diseases of the kidneys. Other cases were chosen as controls, or because fat had been reported in the glomeruli by other observers in the diseases present. Of the autopsies, 30 were from patients suffering from nephrosclerosis and of these 21 (70 per cent) showed fatty changes in the afferent arteries and 12 (40 per cent) in the glomeruli themselves. Among 13 cases of chronic glomerulonephritis, none showed fatty changes in the afferent arteries and 6 had fat, usually in very minute quantities, in the glomeruli. Of 8 cases of acute and subacute glomerulonephritis, 5 showed fat in very fine droplets in the glomeruli. Our miscellaneous group included 1 or more cases each of diabetes mellitus, diphtheria, acute yellow atrophy and cirrhosis of the liver, pneumonia, eclampsia, amyloidosis, abscess of the lung and poisoning with bichloride of mercury and with veronal.

The experiments on dogs were designed to test the theory that a colloidal poison might circulate in the blood in such high dilution as to cause no serious damage to other organs but, as a result of concentration in the glomeruli by loss of water, might injure these structures; and, on the other hand, that a crystalloidal poison might circulate in the blood in sufficiently high dilu-

tion that it could pass through the glomeruli without causing
structural or functional damage and yet injure the tubular epi-
thelium as a result of concentration in the tubules by the absorp-
tion of water. The total blood volume of each animal was esti-
mated and a known amount of the poison was slowly injected
intravenously so that each 100 cc. of the dog's circulating blood
would contain a definite concentration in milligrams of the poison
or in units of toxin. The results are shown in Table I.

TABLE I
*Incidence of Glomerular Fat Following Intravenous Injection of
Various Substances*

Experiment	Total number of animals	Total number with fat in glomeruli	Percentage
Controls	37	4	18.8
Snake venom	20	6	30.0
Streptococcus toxin	6	2	33.3
Staphylococcus toxin	8	2	25.0
Diphtheria toxin	19	5	27.8
Potassium dichromate	11	4	36.4
Uranium nitrate	22	4	18.2
Bichloride of mercury	10	0	00.0
	133	27	

Of the 37 control animals, most of which had been under ether
or nembutal anesthesia for varying periods up to an hour or
more, 4, or 10.8 per cent, showed fat in the glomeruli. Two of
these were suffering from chronic glomerulonephritis with pro-
liferation of the endothelium of the glomeruli and casts in the
tubules. These 2 animals had large quantities of fat in the
glomeruli, most concentrated at the hilus. In the other 2 ani-
mals, 1 of which had pneumonia, the fat was scanty and in the
form of very fine dustlike particles in the walls of the vessels at
the hilus.

The incidence of fatty changes in the glomeruli of those ani-
mals injected with colloidal poisons (snake venom, and strepto-
coccus, staphylococcus and diphtheria toxins), ranged from 25
to 33.3 per cent. The type of fatty changes in the glomeruli of
a dog given minimal doses of diphtheria toxin is illustrated in
Figure 1. The fat is concentrated in the region of the hilus of
the glomerulus and radiates outward in the walls of the capillary
loops. Two of the crystalloidal poisons also produced fatty

changes in the glomeruli, potassium dichromate in 36.4 per cent of the dogs, and uranium nitrate in 18.2 per cent. These include all animals to which these poisons were administered, including those which received more than the optimal dose. On the other hand, none of the dogs poisoned with bichloride of mercury showed fat in the glomeruli although 4 died as a direct result of the action of the poison. Although no very great value is claimed for the statistics of these groups of animals, because they include experiments designed to determine the optimal dose, it may be noted that of 53 dogs injected with colloidal poisons, 15, or 28.3 per cent, showed fatty changes in the glomeruli; while of 43 dogs injected with crystalloidal poisons, only 8, or 18.6 per cent, showed such changes.

DISCUSSION

Fat occurs in the glomeruli in several different forms and locations.

1. In the slighter degrees of this change the fat appears in very fine dustlike droplets in the walls of the capillaries just within the hilus of the glomerulus. The afferent artery is not involved, although we have occasionally seen this form of fat in the juxtaglomerular myoneural apparatus, an observation also made by Goormaghtigh and Handovsky. It is particularly difficult to determine whether the fat in this location is in epithelial or endothelial cells. It is equally difficult to determine whether the fat is in the afferent or in the efferent end of the capillary loops. This is a matter of some importance, as will be pointed out later. This hilar distribution of fat in exceedingly fine particles is characteristic of the fatty changes in the glomeruli in acute infections, such as pneumonia, scarlet fever, peritonitis and glomerulonephritis, and was present in 23 out of the 27 dogs whose glomeruli contained fat.

2. In a more extensive form of fatty change the fat is present in larger droplets, is most concentrated in the region of the glomerular hilus, extends outward along the capillary loops and has much the same distribution as the hyaline thickening of the basement membrane in chronic glomerulonephritis. The afferent arteries are either free from fat or contain only minute quantities and the glomeruli affected are usually well supplied with blood.

This form of fatty change was especially prominent in 2 of our dogs injected with very small doses of diphtheria toxin in 2 cases of chronic glomerulonephritis.

3. Fat was found in the glomeruli in greater abundance in nephrosclerosis (essential hypertension) than in any other condition. In 70 per cent of our cases of this disease fat was present in the afferent arteries (Fig. 2), and in 40 per cent, also in the glomeruli. We have not observed fat in a glomerulus, the afferent artery of which showed only hyperplastic intimal thickening. The fat is in the form of fine or coarse droplets at the periphery of capillary loops that are almost bloodless. Such glomeruli are frequently lobulated so that the droplets of fat form rounded groups, often so compact that it is difficult to determine whether they are intracellular or free in a necrotic mass. The fat in the afferent artery is usually not continuous with that in the periphery of the glomerulus but is separated by a zone in which fat is absent or present in relatively much smaller quantities. However, occasionally an entire glomerulus contains fat. Prym (1904) has illustrated such a case in which "the capillary loops appeared to consist only of fat." Not infrequently the walls of afferent arteries are thick, hyaline and rich in fat while their corresponding glomeruli are well supplied with blood and wholly free from fat. This type of fatty change is illustrated in Figure 2.

4. The epithelium lining the capsular space may contain visible fat, particularly in cases of acute and chronic glomerulonephritis and in acute infections, such as diphtheria and scarlet fever, and in intoxications. It is usually but not invariably associated with fat in the corresponding glomeruli themselves. The most marked example of this type in our series of cases was in a young woman who died from acute hemorrhagic pancreatitis and had an extreme degree of fatty changes in the liver and in the tubular epithelium of the kidneys. In subacute glomerulonephritis fine fat droplets are often present in the cells of the crescents. In some cases of nephrosclerosis small clumps of fatty cells are seen in the capsular spaces. These are epithelial cells desquamated from the glomeruli either as a result of a superimposed glomerulonephritis (Volhard and Fahr) or of necrobiosis of the capillary loops (Jores).

5. Finally, the hyaline scars which occupy the site of glomeruli

that have been completely destroyed often contain fat in fine droplets.

The accumulation of fat in the cells of the glomeruli, as in those of the tubules, is due to injury which renders the affected cells incapable of utilizing fat in the normal manner. The injury may result either from the action of a toxin or some other poison, or from anoxia due to the marked narrowing of the afferent arteries.

In nephrosclerosis the fatty changes in the glomeruli appear to result entirely from ischemia, for in this condition we have not observed fat in glomeruli that were well supplied with blood even though the wall of the afferent artery was hyaline and contained much fat. The presence of fat in the glomeruli in this disease is accompanied by other changes in the capillary loops, ranging from simple collapse to partial or complete hyalinization or even necrosis. Since the blood is the only source of fat, it can accumulate only in glomeruli through which some blood, even if in greatly reduced amount, is still circulating. Under such a condition of anoxia the cells of the glomeruli may acquire fat, perhaps for the same reason that the muscle fibers of the heart become loaded with fat in severe anemias. Why the cells in the periphery of a glomerulus are the first to acquire fat, and the remainder become fatty only in extreme grades of the condition, is not known.

Fatty changes in the glomeruli are much less marked in glomerulonephritis than in nephrosclerosis. In glomerulonephritis the wall of the afferent artery is either entirely free from fat or contains only very minute amounts. Within the glomerulus the fat is most concentrated near the hilus and may be entirely limited to this region. When present in larger amounts it tends to follow the capillary loops radially toward the periphery of the glomerulus. It is generally believed that in this condition the glomeruli are damaged by toxic substances brought to them in the blood stream. A highly dilute colloidal poison in the blood plasma would become more and more concentrated as the blood traverses the capillary loops and would reach its greatest concentration at the efferent ends of these loops. The greatest injury to the cells would occur, and presumably the fatty degeneration would be most marked, at this place. This may account for the concen-

tration of fat in the region of the hilus in acute infections, in glomerulonephritis and in the kidneys of dogs poisoned with minute doses of diphtheria toxin. The latter is believed to be a colloid, albeit of relatively small molecular size, but to which the glomerular filter is either not at all, or only very slightly, permeable. Fat in the glomeruli in diphtheria, scarlet fever, pneumonia, peritonitis and other acute infections is of the fine-droplet, hilar type.

Proof that the fat in capillary walls near the hilus of a glomerulus is in the efferent ends of the capillaries is difficult to establish. It is not present in every glomerulus in a section. It is relatively easy to identify the afferent artery as it enters the glomerulus. Identification of the efferent vessel is far more difficult. In spite of prolonged search we have not been able thus far to find the desired combination of a glomerulus which contains fat in its hilar region and also shows the efferent vessel leaving its hilus. However, in many instances the afferent artery was traced into the glomerulus and the fat was found to be in the walls of capillaries on one or both sides of this vessel and apparently not directly associated with it. This suggests, although it cannot be said to prove, that the fatty changes were in the efferent ends of the capillary loops.

CONCLUSIONS

1. Fatty changes occur in the glomeruli in acute infections such as diphtheria, pneumonia and peritonitis; in acute and chronic glomerulonephritis; and in nephrosclerosis.

2. In acute infections and in glomerulonephritis the fat is most abundant in the immediate vicinity of the glomerular hilus and may be limited to this region. When present in greater abundance it tends to extend radially along the capillary loops, having much the same distribution as the hyalinization and thickening of the basement membrane in chronic glomerulonephritis. In the early stages it consists of very fine dustlike particles; with increase in amount the droplets also increase in size. The afferent artery is free from fat.

3. In nephrosclerosis the walls of the afferent arteries contain much fat which stops at the hilus of the glomerulus. The fat in the glomeruli appears first in the periphery, the central portion

being involved only in those glomeruli in which the fat is very abundant.

4. Fatty changes in the glomeruli in acute infections and in glomerulonephritis are apparently the result of direct injury to the cells by some toxic substance in the circulating blood and concentrated in the glomeruli by loss of water by filtration. In nephrosclerosis the anoxia resulting from the ischemia caused by narrowing of the lumen of the afferent artery induces similar effects.

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DESCRIPTION OF PLATE

PLATE 119

FIG. 1. Photomicrograph from the kidney of a dog given small doses of diphtheria toxin intravenously, with production of acute glomerulonephritis. The afferent artery is seen to be free of fat. That in the glomerulus itself is concentrated at the hilus and radiates outward in the capillary loops. This type of fatty change is also found in some cases of acute and chronic glomerulonephritis in man. Sudan III stain. × 315.
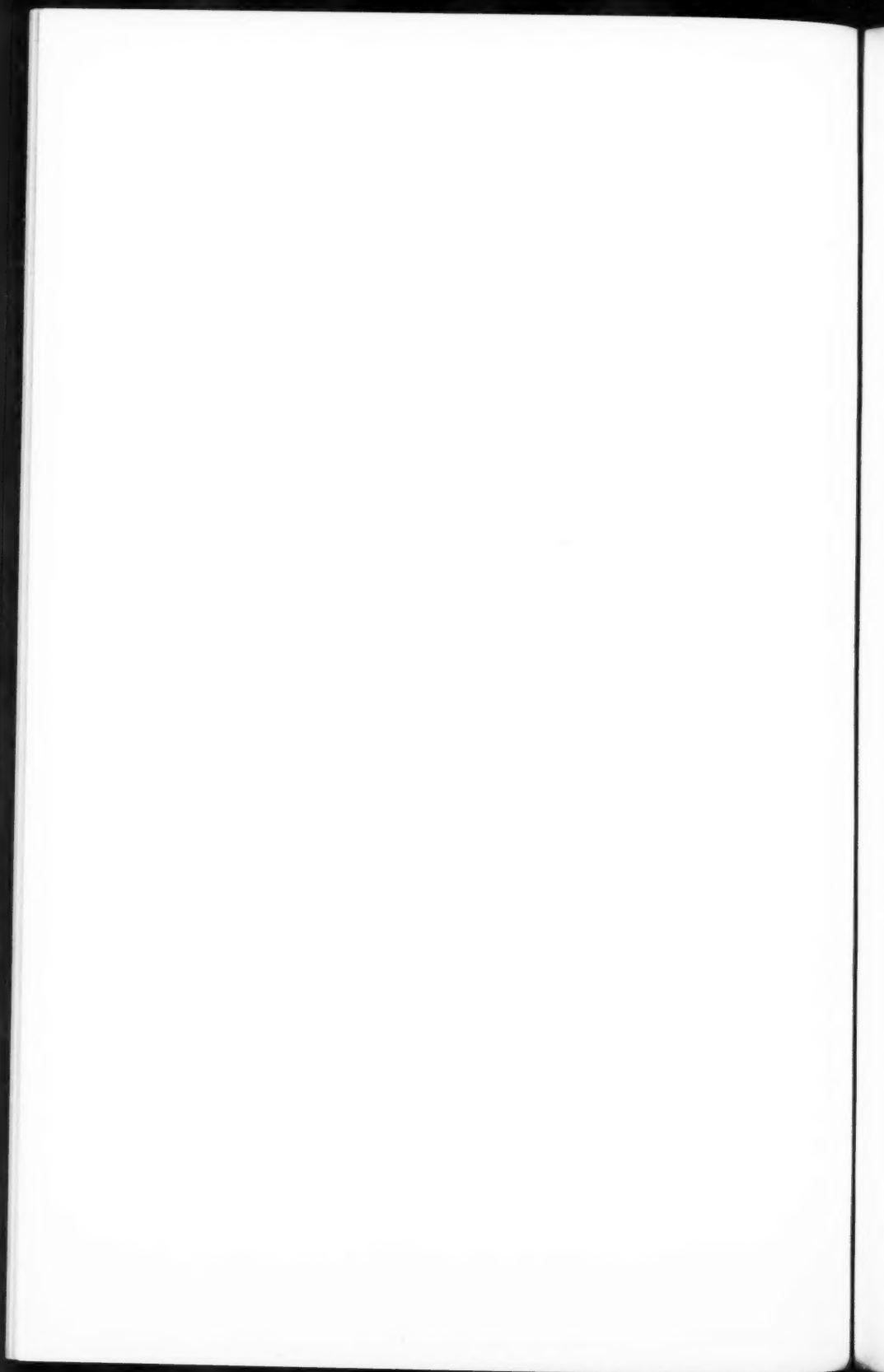
FIG. 2. Photomicrograph showing the type of fatty change seen in the glomeruli in nephrosclerosis. The wall of the afferent artery contains much fat (stained dark) which does not extend into the glomerulus. The fat in the glomerulus is at the periphery. Sudan III stain. × 210.
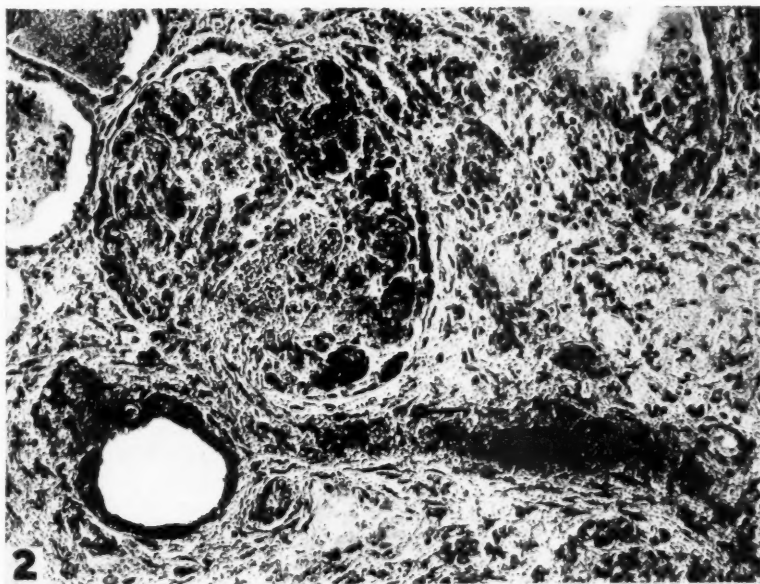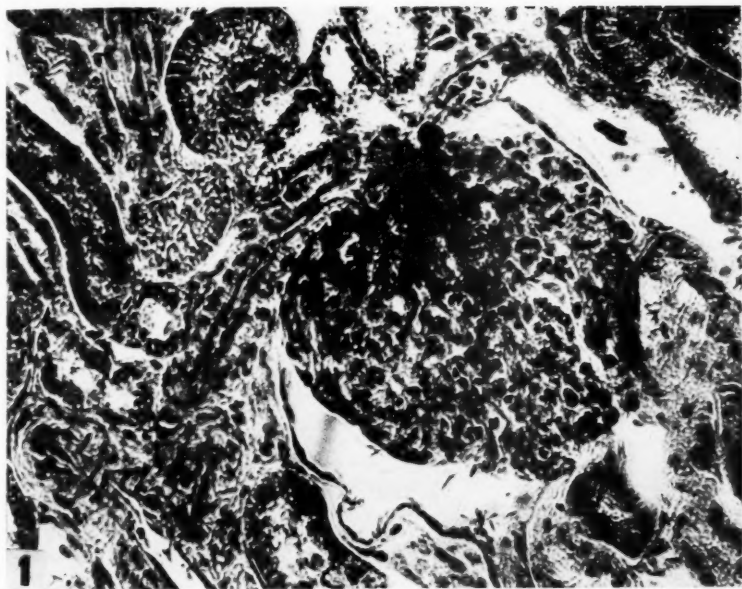
Simonds and Lange   Fatty Changes in Glomeruli

DEGENERATION OF THE ADRENAL CORTEX PRODUCED BY GERMANIN*

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The possibility that germanin (Bayer 205) might injure the adrenal glands was suggested by the findings at autopsy of a patient with pemphigus who had been treated with this drug. As previously described [1] the adrenal lesions were of the type often referred to as primary or cytotoxic atrophy, a type frequently associated with symptoms of Addison's disease. The probability that germanin was a factor in the pathogenesis of this patient's adrenal lesions was strengthened by Tomlinson's [2] report of a similar case. We have failed to find records of similar lesions or of any constant lesions in the adrenal glands of patients with pemphigus who have not been treated with germanin. Preliminary experiments [1] demonstrated that germanin could damage the adrenal cortex, and the present report summarizes the observations on a larger group of animals. In this study we have been less concerned with the general effects of germanin than with ascertaining the type of injury which this therapeutic agent can inflict on the normal adrenal gland.

PROCEDURES

Germanin was administered to 100 guinea pigs, 30 rats, 8 rabbits and 3 dogs, in the form of a 10 per cent solution in freshly boiled distilled water. The drug used was a product of the I. G. Farbenindustrie, marketed for human use. The rats and most of the guinea pigs were injected subcutaneously, the other animals by the intravenous route. All animals were maintained on adequate diets and no adrenal lesions of the types attributed to vitamin deficiency [3] were observed in control animals. The main part of this report summarizes the observations on 90 guinea pigs, most of them young males weighing 250 to 350 gm.

In human therapy germanin is administered in courses of variously spaced injections, often after smaller initial doses to detect unusual sensitivity. The maximum single dose considered

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safe for the human adult is 1.0 gm., or 0.02 gm. per Kg. In these experiments the dose of 0.02 gm. per Kg. was employed as the minimum single dose in serial injections imitating courses used in human therapy, while larger single and serial doses were used to ascertain injurious effects. Table I summarizes the dosages employed for guinea pigs. Group A received single injections of amounts from five to twenty times the minimum dose. Groups B, C and D were given serial toxic doses, variously spaced, and in the case of group D, injected intravenously. The courses for groups E, F and G were planned to imitate therapeutic courses and those of groups H and I were similar but prolonged. In some groups the intervals between injections were varied in order to detect cumulative effects, since germanin is known to be eliminated slowly.[4] Injections were made on alternate days in groups B, E, F, H, and I and at wider intervals in groups C and G. With groups G and I longer rest periods were interposed between series of injections in an attempt to ascertain whether the previously damaged adrenal was more or was less susceptible to injury.

TABLE I
Summary of Dosages and Effects of Germanin

Group	No. of guinea pigs	Doses, gm./Kg.	No. of doses	Intervals (days)	Total gm./Kg.	Total course (days)	No. died	No. with zonal lesions in adrenals
A	12	0.1 −0.4	1	0	0.1 −0.4	1	4	11
B	12	0.03−0.1	*	2	0.2 −0.4	*	7	12
C	8	0.05−0.1	*	8	0.3 −0.5	*	2	8
D	6	0.03−0.4	*	2−20	0.09−0.4	*	2	4
E	13	0.02	5	2	0.1	9	0	3
F	13	0.02	10	2	0.2	19	0	5
G	5	0.02	16	2−20	0.32	100†	0	0
H	16	0.02	11−30	2	0.22−0.6	21−59	7	16
I	5	0.02	20−30	2−20	0.3−0.6	59−79†	1	5
Total	90						23	64

* Number of injections and duration of courses varied.
† Rest periods interposed between courses.

The animals that died and surviving animals, killed from 1 to 21 days following the last injection, were autopsied as soon as possible. Those showing pneumonia or other disease were excluded from the study. It was not feasible to free and weigh the adrenals, as handling them caused confusing artefacts. The kidneys and adrenals were fixed and imbedded together in order to standardize the planes of sections and to permit rough com-

parisons of size. Usually sections of the liver and heart, and
sometimes of other tissues, were prepared. Sections of kidneys,
liver and heart were examined for fat.

RESULTS

The Effects of Single Toxic Doses of Germanin (Table II)

Adrenal cortical lesions were found in 11 of 12 guinea pigs
given single injections of from 0.1 to 0.4 gm. of germanin per
Kg. These were constantly located in the outer part of the
fasciculate zone, sometimes encroaching on the glomerular zone.
The type characterized as an *acute degenerative band* (Fig. 1)
was seen in 4 animals, all dying early after doses of 0.3 and 0.4
gm. per Kg., the established lethal dose for the guinea pig.[5] These
bands, one-third to one-half the width of the cortex, were sepa-
rated from the capsule by only a few groups of small dark cells.
They showed completely disorganized trabeculae, degenerating
cells with pyknotic or fading nuclei, scattered epithelial cells with
rounded borders, and neutrophilic leukocytes. Besides these

TABLE II

Effect on the Adrenals of Single Toxic Doses of Germanin

Guinea pig No.	Dose, gm./Kg.	Died or killed	Day	Adrenal zonal changes	Maximum No. of mitoses per high power field
1	0.4	Died	5	Acute degenerative bands	0
2	0.4	Died	5	Acute degenerative bands	0
3	0.3	Died	5	Acute degenerative bands	0
4	0.3	Died	6	Acute degenerative bands	0
5	0.3	Killed	8	Broad reactive bands	3
6	0.3	Killed	14	Broad reactive bands; more repara- tive activity	8
7	0.2	Killed	8	Like No. 5; narrower	3
8	0.2	Killed	14	Broad reactive bands; more repara- tive activity	8
9	0.2	Killed	21	Advanced repair	20
10	0.1	Killed	8	Narrow, but like Nos. 5 and 7	3
11	0.1	Killed	14	Advanced repair	3
12	0.1	Killed	21	None	2

sharply demarcated bands, many scattered degenerating cells
were seen throughout the cortex.

Lesions characterized as *reactive bands* were seen in animals
which survived doses of 0.1 to 0.3 gm. per Kg. They contained
remnants of degenerating cells, swollen endothelial cells and
macrophages, a few neutrophils, lymphocytes and eosinophils,

and sparse large eosinophilic epithelial cells. Mitotic figures, a few of them in endothelial cells but mostly in epithelial cells, were found throughout the cortex. They were especially numerous in and near the bands. In the older lesions large and sometimes multinucleated epithelial cells were invading the bands from both sides (Fig. 2).

The absence of changes in the adrenals of guinea pig No. 12 should be noted. Since a dose of this size invariably produced zonal lesions it seems likely that the interval of 3 weeks had sufficed for complete healing.

The Effects of Multiple Toxic Doses of Germanin

Twenty guinea pigs were given series of injections with single doses of 0.03, 0.05 or 0.1 gm. per Kg. each. Twelve (group B) were treated intensively, with injections on alternate days, while 8 (group C) were injected 8 days apart. Eleven animals of group B died or were killed because they were moribund, while only 2 of group C died. The difference is especially significant since most of the members of group C were given larger single and total doses.

The character of the zonal adrenal lesions, present in all, varied considerably. Those of 4 animals, dying after intensive series of 3 or 4 injections of 0.1 gm. per Kg. each, were *acute degenerative bands*. These were indistinguishable from those resulting from single injections of 0.3 and 0.4 gm. per Kg. The adrenals of animals treated intensively but with smaller doses showed *mixed degenerative-reactive bands*. An interesting feature was the presence in and near these bands of large cells with bizarre nuclei, some of them certainly abnormal and degenerating mitotic figures. The appearance suggested that acute injury had been superimposed on a phase of repair. Some members of group C had similar lesions, but others presented a different type, the *collapsed band*, which was usually observed in a small gland. These bands were narrow, devoid of epithelium and made up of stromal elements with wide capillaries lined by numerous but flat endothelial cells. Here and there in bands a few lymphocytes and macrophages filled with yellow (lipochrome?) pigment were present. There was no fibrous hyperplasia and the whole appearance suggested the condensation of a previously wider

zone in which repair had been arrested. However, there was some reparative activity at the borders, save in the animals that died.

The Effects of Multiple Small Doses of Germanin

The courses of the drug given animals of groups E, F and G paralleled some of those reported as having been used in human therapy. There were definite zonal changes of the mixed type and some thinning of the cortex in 3 animals given 5 doses of 0.02 gm. per Kg. and in 5 animals given 10 similar injections. Slight degenerative changes were seen in the adrenals of other members of both groups. Figure 3 shows the adrenal lesion of a member of group F. No lesions were observed in the guinea pigs of group G, given 16 widely spaced doses of 0.02 gm. per Kg. each. There were no fatalities in these groups.

The members of groups H and I received from 11 to 30 injections of 0.02 gm. per Kg.; in the case of Group I, with a 20-day rest period after the 15th dose. The 8 fatalities were not determined by total dosage and some followed courses only a little more prolonged than those of group F (see Table III). Adrenal lesions were present in all and every animal given more than 15 doses had collapsed bands, usually in glands which were small. In the smallest glands seen after the longer courses the surviving cortical cells were large and eosinophilic, with large vesicular nuclei (Fig. 4). They resembled the islands of large cells sometimes seen in human adrenal glands showing cytotoxic atrophy. Regenerative activity was noted at the margins of the bands save in the adrenals of some of the animals that died.

Table III presents the data for 13 guinea pigs of groups E, F and H, arranged in order of increasing total dosage. These animals were comparable as to size, sex and survival. All died or were killed 2 days after the final injection.

Evidence for the Cumulative Action of Germanin

The differences in mortality and in character of adrenal lesions in groups B and C are almost certainly attributable to the cumulative action in the intensively treated group B and the longer time for the elimination of the drug between doses in group C. Other animals, paired as to dosage and differing only as to the spacing of injections, showed similar differences. For instance,

guinea pig No. 20 (Table III) received 16 injections of 0.02 gm. per Kg. on alternate days, or in 31 days, while several animals of group G received the same dosage in 100 days. The first animal died and its adrenals had collapsed bands with little regeneration, while the other animals had normal adrenal glands.

TABLE III

Adrenal Lesions After Serial Small Doses of Germanin*

Guinea pig No.	No. of doses	In days	Died or killed	Cortical zonal lesions in the adrenal glands†
13	5	9	Killed	Narrow band with a few degenerating cells
14	10	19	Killed	Band with mixture of degeneration and repair
15	11	21	Died	Similar; less repair
16	12	23	Died	Collapsed band; slight repair
17	14	27	Died	Mixed band with regions of collapse
18	15	29	Killed	Collapsed band; some repair
19	15	29	Killed	Collapsed band; good repair
20	16	31	Died	Collapsed band; no repair
21	20	39	Died	Collapsed band; no repair
22	20	39	Killed	Collapsed band; slight repair
23	25	49	Killed	Similar; fair repair
24	26	51	Died	Collapsed band with marked acute degeneration in adjacent cortex
25	30	59	Killed	Collapsed band; slight repair

* Single doses of 0.02 gm. per Kg. were injected subcutaneously on alternate days. All animals were comparable as to initial weight and sex. All died or were killed 1 or 2 days after the final injection.
† With few exceptions these adrenal glands were small in comparison to the size of the animal.

Character of Adrenal "Atrophy" Caused by Germanin

Decrease in size of the adrenal glands was seen mainly in animals given repeated small injections and was especially marked in those receiving 20 or more doses. The process certainly was not a simple atrophy but resulted from the disappearance of parenchymatous cells, collapse of the less damaged stroma and lagging repair. A striking similarity to the processes in so-called acute yellow atrophy of the liver was noted by Wells, Humphreys and Work.[1] In both cases, if the process is to be characterized as "atrophy," the qualifying term of "cytotoxic" is warranted.

Miscellaneous Toxic Effects of Germanin on Guinea Pigs

Losses of weight and strength were the most obvious general manifestations, and after prolonged courses losses of from 20 to 40 per cent of the initial weight were noted. Since most of the animals were young, even the lesser losses of weight were significant.

In general, germanin is not to be classed as a steatogenic poison nor one causing fatty degeneration, and fatty changes were usually insignificant. There were, however, a few large (800 to 1150 gm.) animals, all given toxic doses. While the other effects were similar, there was one striking difference. The kidneys, livers and hearts of these mature guinea pigs showed profound fatty changes. Since young animals had been given similar doses without showing fatty changes, the difference in age seemed to be the only explanation.

Clinical observations and previous experimental studies have stressed the injurious effects of germanin on the kidneys. Renal lesions were common in this series. Large doses caused profound tubular degeneration with hydropic changes and sloughing of epithelium, and small serial doses usually caused some tubular damage. However, the kidneys frequently showed marked regenerative hyperplasia. In some animals with adrenal lesions they were essentially normal. Either they were less susceptible to injury or had recovered more rapidly.

Changes in other organs were not conspicuous. Toxic doses caused some degenerative changes in the liver, but these were not spectacular. In a few cases the lungs showed edema or small hemorrhages. The bone marrow, examined only a few times, was not unusual. The same was true of the spleen, pancreas, thyroid and lymph nodes. The brain was not examined.

Effects of Germanin on Rats, Rabbits and Dogs

Too few animals were studied to permit more than general observations. Adrenal lesions similar to those of guinea pigs were observed in both rats and rabbits. After toxic doses, rats frequently had petechiae in the skin and in mucous and serous membranes, and large subcutaneous hemorrhages at the sites of injections. No such hemorrhagic tendency was observed in rabbits and guinea pigs. No lesions were observed in 3 dogs, all of them young animals, given small series of injections with minimum doses.

SUMMARY AND DISCUSSION

This study confirmed previous observations on the toxic and lethal effects of germanin, its cumulative action and the capacity of toxic doses to injure the kidneys. It established the fact that

germanin has a selective action in damaging the adrenal cortex, equal to or surpassing its ability to injure renal tubular epithelium. It is impossible in most cases to estimate the relative importance of renal and adrenal damage in causing death of the animals. However, in a few instances, animals that died had zonal lesions of the adrenals and practically normal kidneys.

Even more important than the fact that large doses of germanin may cause extensive destruction of the adrenal cortex is the fact that small doses, comparable to those used in man, may cause similar though less extensive lesions. It is significant that the zone where the damage occurs is the zone of the cortex where growth is normally most active. This undoubtedly was a factor in the decrease in size or "atrophy" noted after many small doses. However, with normal animals and with the dosages used, the destruction of the cortex was never complete, and in surviving animals the capacity to regenerate was not completely lost. There was no evidence that sensitization to germanin played a part in causing the adrenal lesions of these animals.

As to cytotoxic atrophy of the adrenal glands in man, it seems unlikely that germanin can be responsible for more than rare cases. The drug is little used save in the treatment of African sleeping sickness, pemphigus and a few other cutaneous diseases. Talbott, Lever and Consolazio [6] questioned the importance of the rôle of germanin in the human cases cited,[1,2] since they found chemical changes in the blood of patients with pemphigus which pointed to adrenal insufficiency. However, their observations may have other significance. It is not impossible that a functionally abnormal adrenal gland might have an increased susceptibility to a toxic agent which acts selectively to injure the adrenals. Also, there is still the possibility that drug sensitization may play a part, a possibility not excluded by the failure to demonstrate sensitization in one animal species. It should be noted that lesions resembling cytotoxic atrophy have not been reported in patients with pemphigus who have not been treated with germanin. In fact there seem to be no constant or specific adrenal lesions.

The demonstration that one agent used in the therapy of human disease can consistently injure the adrenal cortex of experimental animals and that it almost certainly has caused cytotoxic atrophy of the adrenal glands of human beings is important in

itself. Even more important, however, is the fact that it empha-
sizes the necessity for systematic morphologic studies in this
period of increasing use of chemotherapy. It proves that it is
not enough to examine the large viscera. Germanin has been in
use for 20 years, yet we have failed to find mention of examina-
tion of the adrenal glands save by gross inspection.

CONCLUSIONS

Germanin in toxic doses consistently produces zonal degenera-
tion of the adrenal cortex of small laboratory animals.

Small serial doses of germanin, comparable to those in thera-
peutic use in man, may produce similar but less intense adrenal
cortical lesions.

It is probable that germanin has occasionally caused cytotoxic
atrophy of the adrenal glands in patients with pemphigus.

It is possible that other therapeutic agents may have a similar
selective action and a capacity to injure the adrenal cortex.

NOTE: This investigation was carried out under the direction of H. Gideon
Wells, to whom we are grateful for advice and criticism.

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DESCRIPTION OF PLATES

PLATE 120

FIG. 1. Acute zonal degeneration of the adrenal cortex of a guinea pig dying 1 day after a course of 4 injections of 0.1 gm. of germanin per Kg. Similar lesions were produced by single doses of 0.3 and 0.4 gm. per Kg. The band involves nearly one-half of the cortex and encroaches upon the glomerular as well as upon the fasciculate zone. There are many degenerating cells throughout the cortex and polymorphonuclear leukocytes invade the band. × 140.

FIG. 2. Repair of zone of degeneration in the adrenal of a guinea pig killed 21 days after a single injection of germanin of 0.2 gm. per Kg. There are many mitotic figures. These are most numerous near and in the band. × 140.
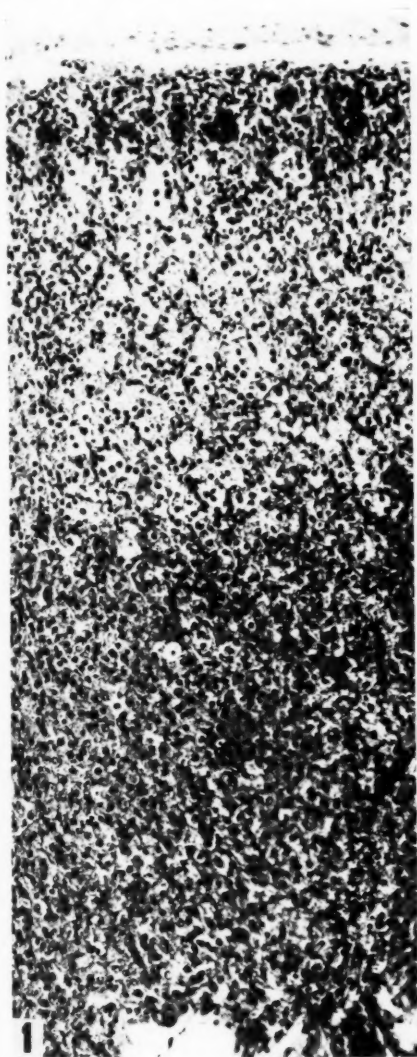
PLATE 120

Humphreys and Donaldson
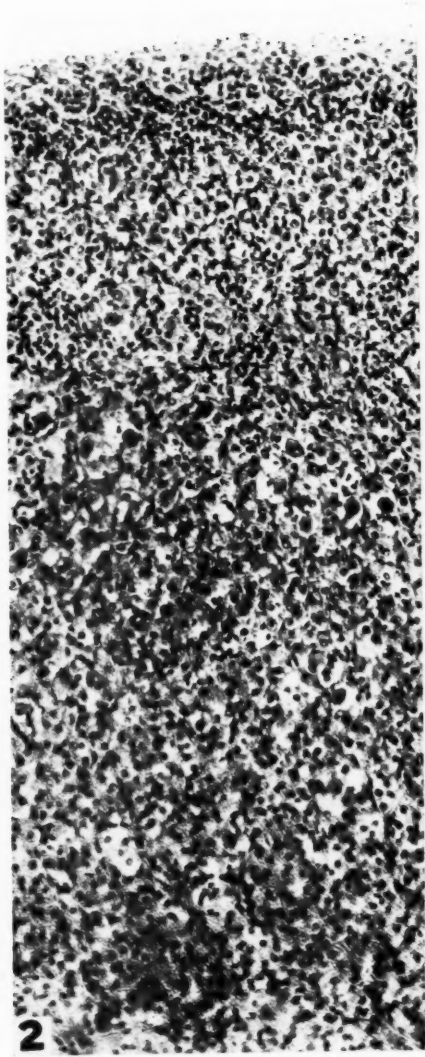
Adrenal Lesions from Germanin

PLATE 121

FIG. 3. Mixed degenerative-reparative changes in the adrenal gland of a guinea pig killed 2 days after a course of 10 injections of germanin, of 0.02 gm. per Kg. each, given on alternate days. The cortex appears thinner than it is normally. × 140.

FIG. 4. Collapsed cell-poor band with marginal regeneration in the adrenal of a guinea pig killed 8 days after a course of 30 injections of germanin of 0.02 gm. per Kg. each, given on alternate days. The thinness of the cortex is not an artefact as the plane of the section is the same as in Figures 1 to 3, and this animal weighed 100 gm. more than any of the other three guinea pigs. × 140.
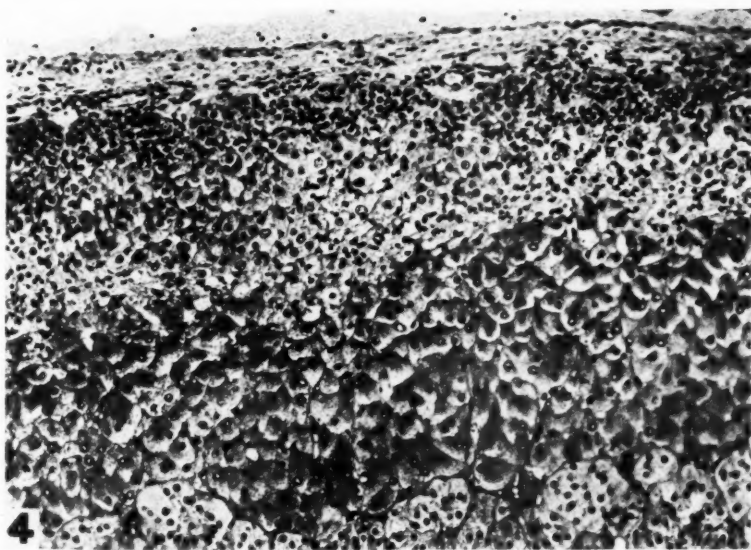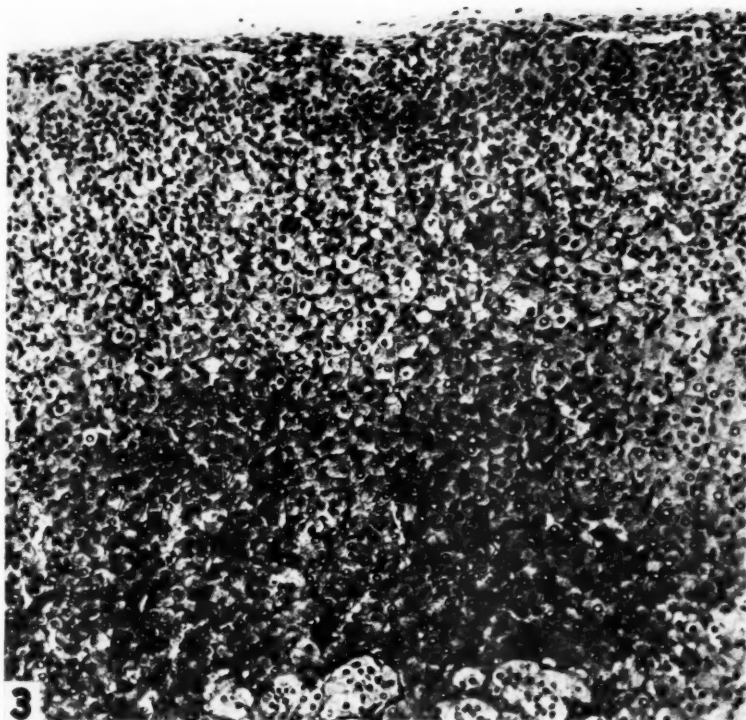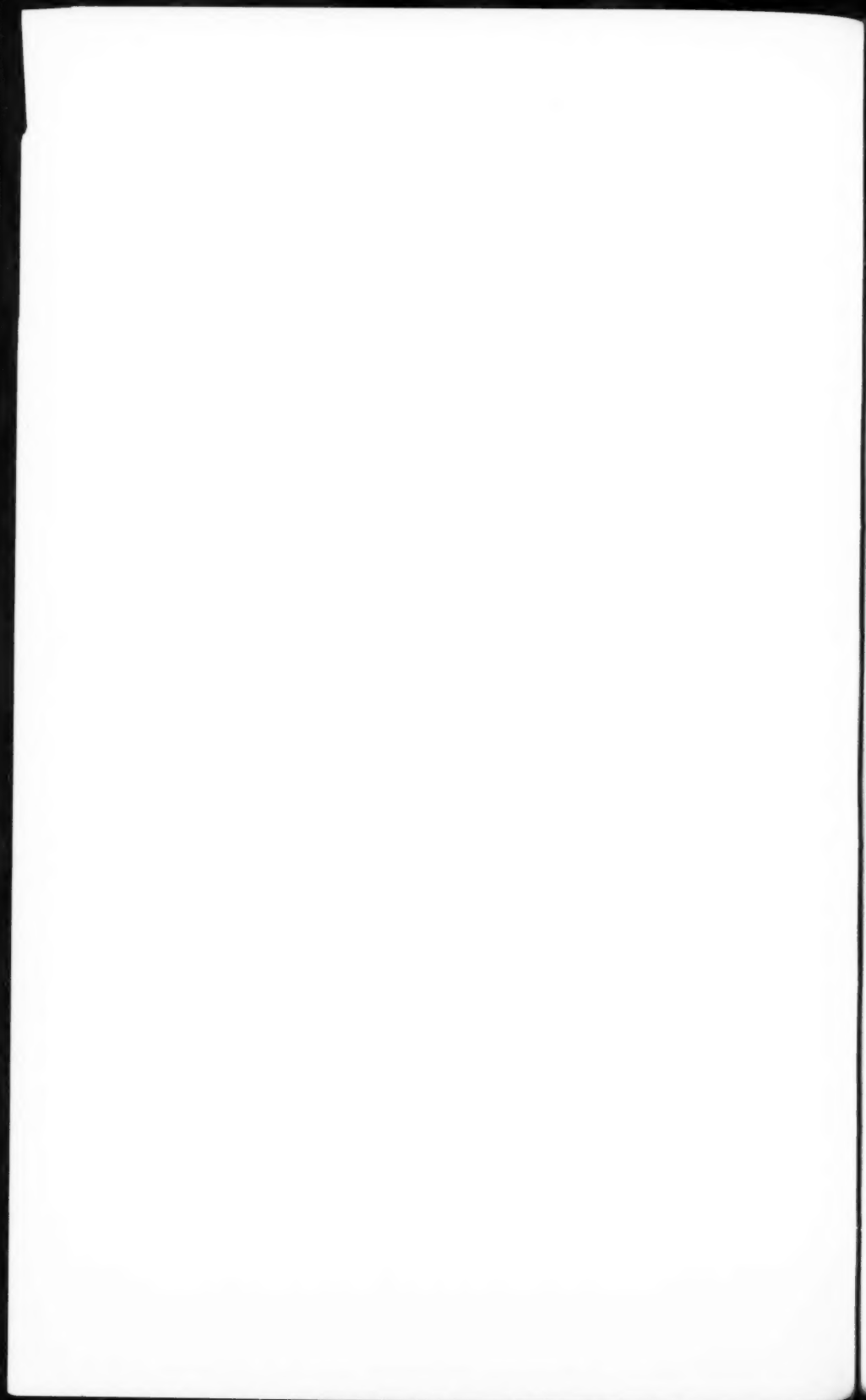
ACUTE LOCAL ANAPHYLACTIC INFLAMMATION OF THE LUNGS*

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The phenomenon of acute local anaphylactic inflammation is not clearly understood, either as to its mechanism of development or its relationship to immunity. Its specificity is generally agreed upon, but the type and source of antibodies are still uncertain. Although there is evidence that the local anaphylactic reaction results from the union of antigen with precipitins in the blood and tissues, some investigators attribute it to a specifically changed reaction-capacity of tissues and suggest that this may be entirely independent of demonstrable antibodies, either in the blood or tissues. Some also regard the entire process as harmful, to be eliminated whenever possible, whereas others consider it as ordinarily beneficent, although occasionally injurious.

A part of the uncertainty relating to the entire problem has come from the use of complex antigens, particularly bacterial cultures, vaccines, or animal proteins containing several antigenic components. Furthermore, these materials have usually been injected into solid tissues, such as the skin, joints, kidneys, heart and aorta—organs in which interstitial, parenchymal and vascular lesions cannot be readily differentiated. Inasmuch as there is considerable evidence that the primary reaction of local anaphylaxis is vascular, it is desirable, in order that the primary lesions may not be obscured by secondary effects, to study its development in a vascular organ containing a minimal quantity of interstitial and parenchymal tissue. The lungs of rabbits, because of their vascularity, seem well suited for such a study, especially since they play such an important part in the development of the anaphylactic reaction in this species. They afford an excellent opportunity, furthermore, for the nontraumatic introduction of the antigen into the air passages where the reaction can develop as a surface phenomenon on a "blood-tissue"

777

barrier; *viz.*, the membrane which lines the alveolus and separates it from the lumen of the intra-alveolar capillary.

Only a few investigators have made histological studies of anaphylactic inflammation in the lungs. Earlier workers [1,2] observed the development of pneumonitis in guinea pigs sensitized to a foreign serum following inhalation of a spray of the serum; Schlecht and Schwenker [3] described pulmonary hemorrhages and foci of acute alveolitis appearing as early as 6 hours after inhalation. Opie [4] injected 0.2 cc. of horse serum through the thoracic wall into the lung of an immunized rabbit and found that it caused "localized consolidation with leucocytes and edema surrounding a central focus of necrosis," whereas, "the same antigen injected into a normal rabbit was absorbed from the lung with no noteworthy change." Fried [5] injected horse serum directly into the trachea of normal and of sensitized rabbits and noted in the latter an intensified inflammation of the lungs, characterized by edema, hemorrhage, leukocytic infiltration, deposition of fibrin, consolidation and necrosis.

The following experiments were performed with the object of studying histologically the pulmonary lesions which might develop in normal and protein-sensitized rabbits following the entrance of a solution of protein into the lungs.

MATERIALS AND METHODS

Rabbits from a source carefully guarded against snuffles were made hypersensitive by several subcutaneous injections, at intervals of from 5 to 7 days, of a solution of dried egg white or of crystalline egg albumin (crystallized three times). The latter substance is a relatively homogeneous purified antigen which is but slightly irritative to normal tissues. For some of the tests a solution of partially purified egg albumin, representing the albumin fraction after the first precipitation with ammonium sulfate, was used. This is referred to as albumin precipitate. The rabbits, when adequately sensitized, as shown by an intradermal injection of 0.1 cc. of a 2 per cent solution of the protein, were paired with normal rabbits and a solution of either crystalline egg albumin or of the albumin precipitate was instilled simultaneously into their nostrils. (It is well known that light fluids, when introduced into the nostrils of unanesthetized rabbits, flow

readily down the trachea into the lungs. This obviates the necessity for intratracheal injection or for direct injection through the thoracic wall with the complicating elements of surgical trauma, hemorrhage, shock or interference by anesthesia.) At varying intervals the animals were killed by air embolism and the lungs fixed *in situ* with a Zenker-formaldehyde solution. Celloidin sections were prepared and stained with hematoxylin and eosin. In some instances rabbits were sensitized passively by the intravenous or intraperitoneal injection of serum from rabbits hypersensitive to crystalline egg albumin and the effects following instillation of the solution of protein into their lungs observed. In some of the animals, also, the precipitative titers, as determined by the collodion particle agglutination method,[6] were determined at the time of instillation of the protein solution.

RESULTS
Findings in Normal Rabbits

Sixteen normal rabbits were used as controls and their lungs examined from 4 to 48 hours after intranasal instillation of the protein solutions. Eleven of the animals were killed 24 hours after the instillation. The effects of the entrance of the egg albumin into their lungs were for the most part slight. Despite the fact that several blocks of tissue were taken from each lung, and that an effort was made to select the most abnormal looking areas, most of the sections showed no significant change. An occasional slight area of acute alveolitis was seen in a few instances, with an associated minimal edema. Acute hemorrhage was never seen and the perivascular lymphatics usually appeared normal. There was no phlebitis or arteritis and no thrombosis. Almost all sections were described as normal and inflammation, when present, was minimal. Crystalline egg albumin or a solution of dried egg white, therefore, may be regarded as practically nontoxic in the amounts and concentrations that entered the lungs of these normal rabbits.

Findings in Rabbits Sensitized Against Egg Albumin

Nineteen hypersensitive rabbits were treated simultaneously with the controls. The effect of the entrance of the protein solutions into their lungs, however, was strikingly different, being

characterized by acute edema, alveolitis, bronchitis and pneumonic consolidation (Figs. 1 and 2). The severity of the reaction depended essentially upon the degree of sensitivity of the animals, the amount of material which apparently entered the lungs and the length of time after its instillation. The lymph flow was markedly increased (Fig. 3) and at times hemorrhage occurred, both into lymphatic spaces and into alveoli (Fig. 4). Acute arteritis and phlebitis were present in some animals, with, in some instances, mural thrombosis in small arteries and veins (Fig. 6). Infarction also occasionally developed. The effect was as if the nontoxic solution of protein had become toxic, at times extremely so. Thus, the primary and outstanding effect was upon blood vessels and was made evident particularly by increased capillary permeability. Only when the effect was more intense did arteritis, phlebitis, infarction and marked pneumonic consolidation occur.

Findings in Rabbits Passively Sensitized

An attempt was made to ascertain whether the pulmonary lesions which appeared so strikingly in actively sensitized animals could be demonstrated also in those passively sensitized. Sera from several rabbits which were strongly hypersensitive to crystalline egg albumin were pooled and 50 cc. were injected intravenously into two normal rabbits (P3 and P4). At the end of 24 hours the animals were found to be definitely hypersensitive to egg albumin and samples of their sera were taken. A solution of precipitated egg albumin was then instilled intranasally and, at the end of 24 hours, a second sample of blood was taken, the animals were sacrificed and their lungs prepared for histological study. The two samples of sera, with serum from a normal animal as a control, were tested against collodion particles to which crystalline egg albumin had been adsorbed. The precipitative titers of both hypersensitive rabbits showed a decline from 1:1920 to 1:120 as a consequence of the intranasal instillation of the albumin and its combination with antibody in the lungs. Sections of the latter (Figs. 7 and 8) showed acute edema, deposition of fibrin within alveoli, early acute alveolitis and hemorrhage, with erythrocytes present in dilated perivascular lymphatic spaces and alveoli.

Two additional rabbits, with two controls, were similarly treated (P6 and P7) and sacrificed at the end of 48 hours. Sections from the lungs of the normal rabbits showed nothing unusual in one and only a few tiny areas of acute alveolitis in the other. In the sensitized animals, however, the pulmonary inflammation was intense, being characterized by marked acute focal alveolitis, dilation of the perivascular lymphatic spaces and accumulations of mononuclear cells and masses of fibrin in the alveolar spaces.

Inasmuch as passive sensitization in the preceding experiments was accomplished by way of the blood stream, an attempt was made to produce it directly within the alveolar spaces. Fox [7] has shown that immune sera, when introduced into the lungs by way of the bronchi, are absorbed but slowly into the blood stream. This is due, presumably, to the slow diffusibility of the larger globulin molecules. Ten cc. of serum from a rabbit hypersensitive to crystalline egg albumin were instilled into the nostrils of a normal rabbit. Twenty minutes later 2 cc. of a 4.4 per cent solution of albumin precipitate were then instilled intranasally. The animal was sacrificed 30 hours later. Sections showed a more marked acute edema and focal alveolitis than were seen in rabbits P3 and P4, with many polymorphonuclear leukocytes centered around eosinophilic masses (precipitate?). This finding would suggest an antigen-antibody reaction occurring largely on or within the alveolar walls, with the increased capillary permeability and acute inflammation ensuing.

It is obvious, therefore, that the local anaphylactic reaction is essentially the same, whether it occurs in rabbits actively or passively sensitized. This must mean that the determining element in the reaction is humoral, presumably the anaphylactic antibody and not primarily a changed reaction-capacity of the tissues.

DISCUSSION

These findings corroborate those of others that, whether in general or local anaphylaxis, much of the reaction is vascular and manifests itself as an increased capillary permeability as the result of specific injury to endothelium. Moon [8] has recently called attention to the striking similarity between the circulatory

disturbances seen in anaphylactic shock and in experimental or clinical traumatic shock and has emphasized the point that in anaphylaxis "the capillary endothelium is a chief point of injury." The injury occurs, presumably as a result of the union of antigen and antibody within the sensitized endothelium. Manwaring, Chilcote and Hosepian,[9] and Petersen and Levinson [10] have shown that in anaphylactic shock the endothelium may be damaged to the point of allowing erythrocytes to pass through. Zander [11] has shown recently, by means of the application of a partial vacuum to the skin of rabbits, that allergic inflammation leads to an increased tendency to capillary hemorrhage or, as he calls it, an increased capillary fragility. Direct evidence of the effect of an antigen-antibody reaction upon vascular permeability is furnished in the experiments of Abell and Schenck.[12] These observers studied the action of horse serum introduced into the moat of an ear chamber in rabbits sensitized to horse serum. They observed contraction of arterioles, with stoppage of circulation, an increased tendency of leukocytes to adhere to the endothelium and passage of leukocytes in large numbers through the walls of capillaries and venules. Leukocytes, at times, also formed clumps large enough to cause embolic blockage in capillaries and venules. With repeated introduction of horse serum into the moat, even extravasation of erythrocytes and endothelial destruction occurred. Rich and Follis [13] more recently have concluded that, as a result of studies of the Arthus phenomenon in the corneas of sensitized rabbits, "the sensitivity that determines necrosis appears to be limited to the blood vessels, and especially to the endothelium" whereas "the cells of the tissues at large are not themselves sensitized."

The exact cause of the increased capillary permeability is uncertain. Lowered oxygen tension with resulting asphyxia of the vessel wall is usually considered of great importance as a cause of such a change. It is possible that this may occur also when antigen and precipitin combine on or within endothelial cells. The interference with cellular respiration might be manifested quickly by the increased permeability which is so conspicuous a feature of the anaphylactic reaction. A final answer cannot be given, however, until such intracellular precipitation can be demonstrated in living endothelium.

SUMMARY

Rabbits made hypersensitive (actively or passively) to puri-
fied egg albumin, and normal controls, were given simultaneous
intranasal instillations of a solution of purified egg albumin. They
were sacrificed at varying intervals, the lungs were fixed *in situ*
in a Zenker-formaldehyde solution and sections were stained with
hematoxylin and eosin.

In the normal animals pulmonary inflammation was minimal
and frequently indiscernible. In the hypersensitive rabbits, how-
ever, the entrance of egg albumin into the lungs engendered the
development of acute pneumonitis, characterized by edema, alve-
olitis, bronchitis and pneumonic consolidation. The perivascular
lymphatics were dilated and contained many erythrocytes. Acute
arteritis and phlebitis occurred at times and mural thrombosis
was occasionally seen. The findings were essentially identical,
whether the rabbits were actively or passively sensitized.

The experiments indicate, therefore, that the primary effect of
the antigen-antibody reaction in the lungs was an increased capil-
lary permeability, followed later by severer vascular injury. They
show, furthermore, that the effect is due to a humoral element,
presumably the anaphylactic antibody, rather than to a changed
reaction-capacity of the tissues. The intensity of the reaction
depends, apparently, upon the varying intensities of the antigen-
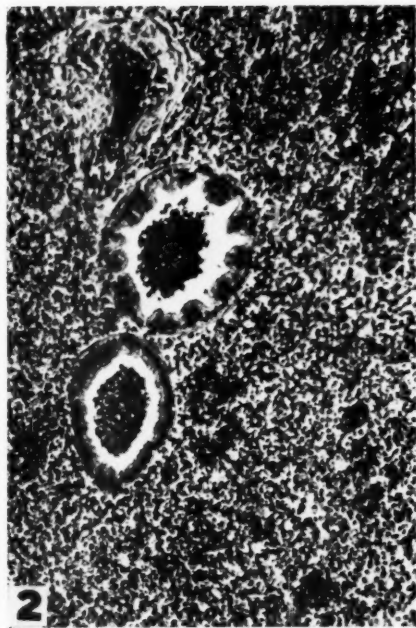antibody union.

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DESCRIPTION OF PLATES

PLATE 122

FIG. 1. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of 10 per cent crystalline egg albumin. Acute edema and alveolitis are present. × 125.

FIG. 2. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of 5 per cent crystalline egg albumin. Taken from an area showing acute bronchitis and bronchopneumonia. × 125.

FIG. 3. From the lung of a rabbit actively sensitized against crystalline egg albumin 8 hours after intranasal instillation of crystalline egg albumin, showing marked dilation of a perivascular lymphatic space. × 130.

FIG. 4. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of crystalline egg albumin. The increased vascular permeability is shown by the presence of erythrocytes in the perivascular lymphatic space. × 290.
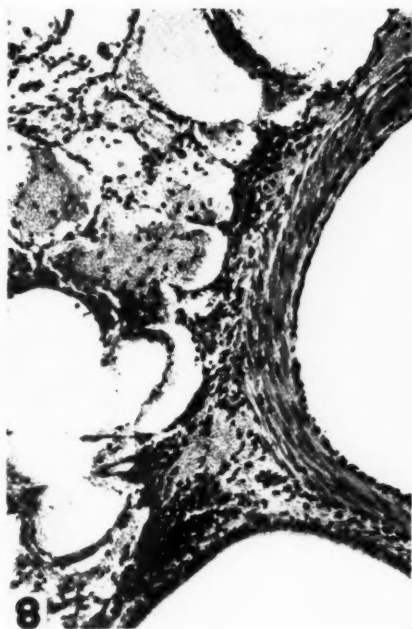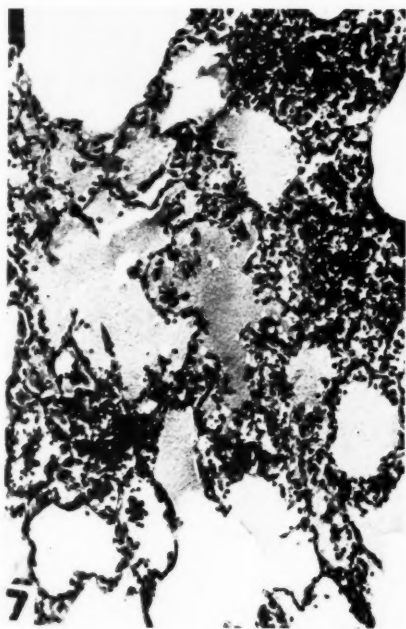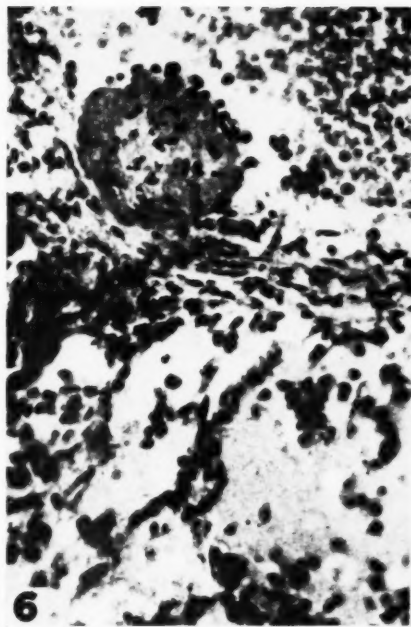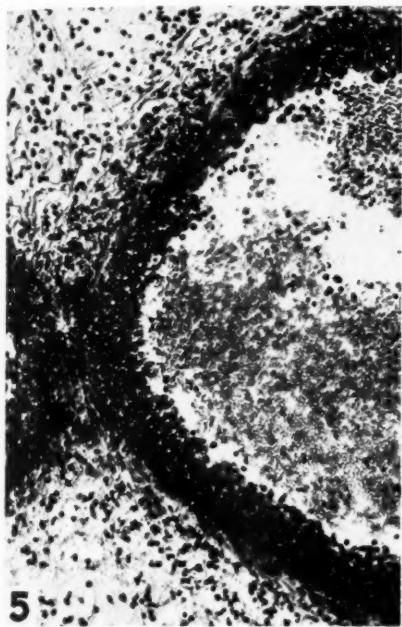
Anaphylactic Inflammation of the Lungs

FIG. 5. From the lung of a rabbit actively sensitized against dried egg white scales 24 hours after intranasal instillation of a solution of dried egg white. Acute phlebitis and acute lymphangitis are seen. × 160.

FIG. 6. Photomicrograph from the lung of the same rabbit used for Figure 1, demonstrating an early mural thrombus in a pulmonary vein. × 275.

FIG. 7. Photomicrograph of the lung of a rabbit passively sensitized to crystalline egg albumin 24 hours after intranasal instillation of albumin precipitate. Acute pulmonary edema and acute alveolitis are seen. × 120.

FIG. 8. Photomicrograph of the lung of the same rabbit shown in Figure 7 to illustrate acute edema and acute hemorrhage into the alveolar and perivascular lymphatic spaces. × 130.
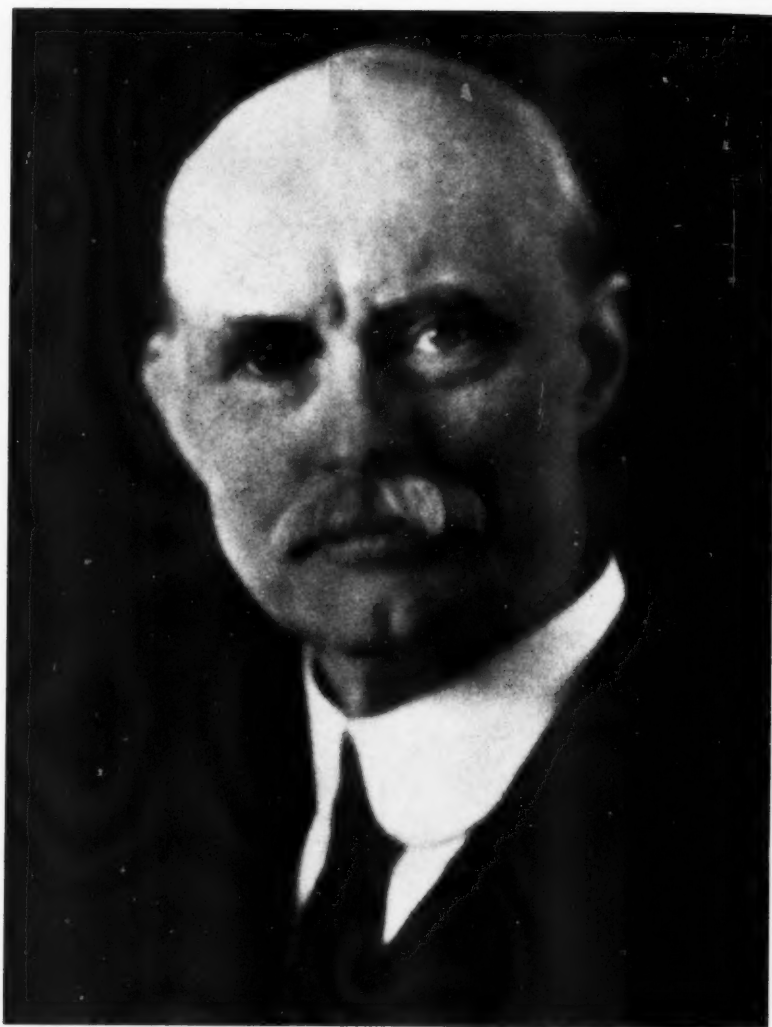
PLATE 123

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Anaphylactic Inflammation of the Lungs
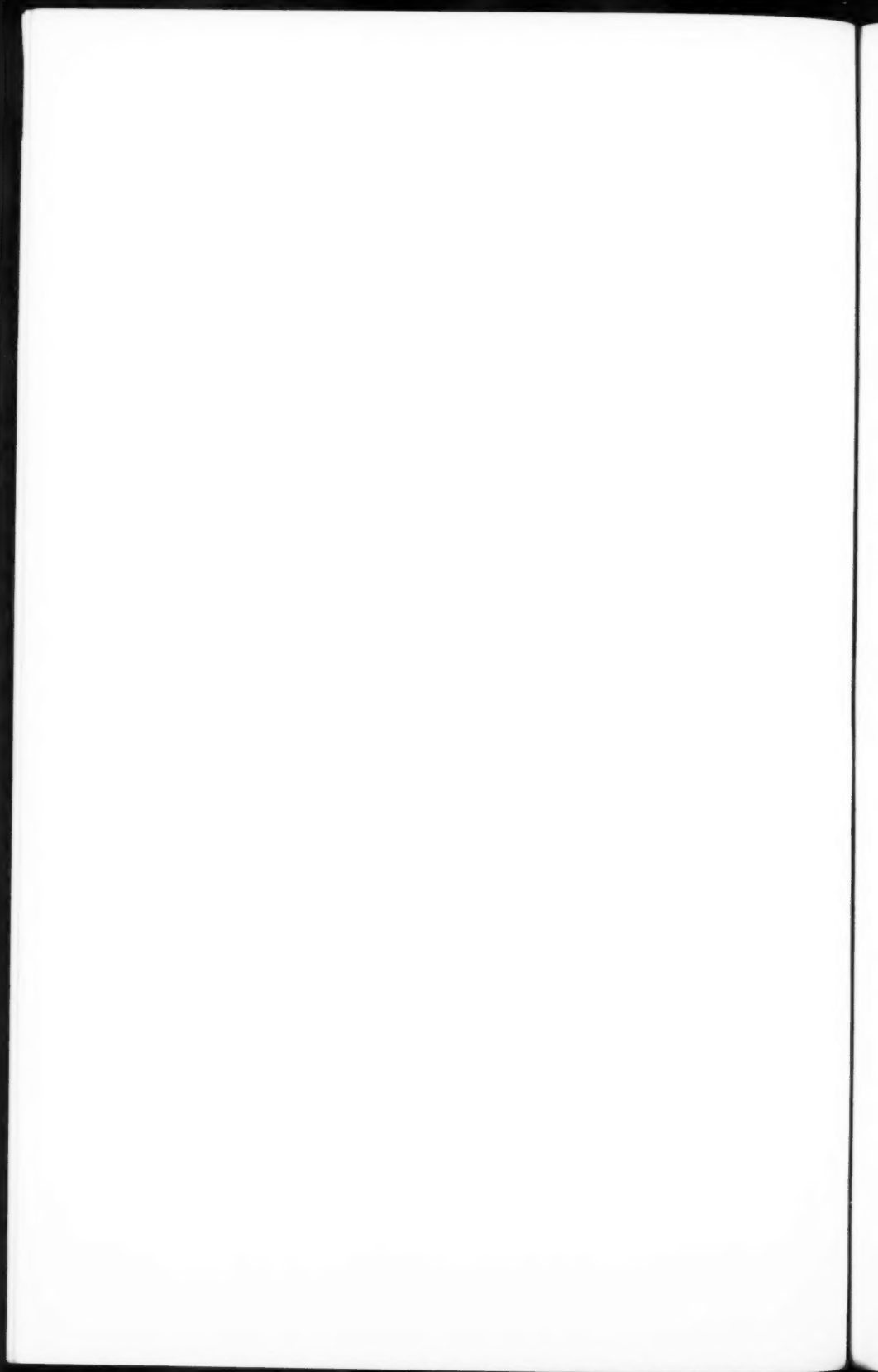
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FRANK BURR MALLORY
(1862-1941)